Spring 1982

MORPHOLOGICAL AND ISOZYMAL VARIATION IN THE SEA-STAR ASTERIAS FORBESI(DESOR)

CHARLES H. TOWLE JR.

INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

University Microfilms International
300 N. Zeeb Road
Ann Arbor, MI 48106

Towle, Charles H., Jr.

MORPHOLOGICAL AND ISOZYMAL VARIATION IN THE SEA-STAR ASTERIAS FORBESI(DESOR)

University of New Hampshire PH.D. 1982

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages ✓
2. Colored illustrations, paper or print ✓
3. Photographs with dark background ✓
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Other _____

MORPHOLOGICAL AND ISOZYMAL VARIATION IN THE SEA-STAR *ASTERIAS forbesi* (DESOR)

BY

Charles H. Towle, Jr.
M.S., Middlebury College, 1967

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
in
Zoology

May, 1982

This dissertation has been examined and approved.

Charles W. Walker

Dissertation Co-chairman, Charles Walker
Associate Professor of Zoology

Yun-Tzu Kiang

Dissertation Co-chairman, Yun-Tzu Kiang
Associate Professor of Plant Science
and Genetics

John H. Dearborn

John Dearborn, Professor of Zoology
University of Maine, Orono, Maine

Larry J. Harris

Larry Harris, Associate Professor of
Zoology

E. Tillinghast

Edward Tillinghast, Associate Professor
of Zoology

4-23-82

Date

DEDICATION

This work is dedicated to my children, Jennifer and Christopher Towle.

ACKNOWLEDGEMENTS

A number of people have contributed in a variety of ways to the preparation of this dissertation and to the research on which it is based. Special thanks are due to Professors Charles Walker, Yun-Tzu Kiang, and Edward Tillinghast for assuming an inordinate amount of responsibility in the direction of my program, and to Professor John Dearborn for his interest and enthusiasm and his suggestions concerning collection sites. The discovery of the interesting population of animals in the salt pond at Blue Hill Falls is attributed to Professor Dearborn. I likewise thank Professor Larry Harris for his interest and enthusiasm. His positive attitude was a sustaining influence during the latter stages of thesis preparation. Wayne Lord, Frank Smith, Fred Thurburg, and Gail Zamarchi helped with the collection of specimens; their contribution is gratefully recognized. I thank Emery Swan for his patience and his faith in me, and appreciation is expressed to Dick Lewontin and John MacArthur for support during a difficult period in my life.

I am grateful for financial support provided by the University of New Hampshire Zoology Department, for two summer fellowships provided by the University Graduate School, and for travel funds made available to me by the University Marine Program. I am indebted to Marlboro College for the awards of two Shell Faculty Assists which helped me to initiate my research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

MORPHOLOGICAL AND ISOZYMAL VARIATION IN
THE SEA-STAR, ASTERIAS forbesi (DESOR)

BY

Charles H. Towle, Jr.
University of New Hampshire, May, 1982

Few examples of geographically-related isozymal variation exist for echinoderms and no such studies are available specifically for sea-stars. The present work considers the existence of morphological and isozymal variation among geographically separated populations of Asterias forbesi, a common sea-star found on the East Coast of North America. Animals studied in this investigation were collected from seven coastal populations located between Beaufort, North Carolina and St. Ann's Bay, Nova Scotia. Each animal was examined for calcification of the carinal ossicles and variation in banding patterns of the enzymes xanthine dehydrogenase (XDH) and cytoplasmic malate dehydrogenase (MDH) resident in the pyloric caeca. Mean weights of carinal ossicles from specimens collected at each location differ significantly ($p \leq .05$) from each other. Both XDH and MDH display multiple phenotypes; phenotypic frequencies vary progressively in the samples collected, northward from Beaufort, North Carolina to St. Ann's Bay, Nova Scotia. XDH frequencies correlate positively with mean annual range in sea-water

temperature, while MDH frequencies correlate with latitude. One MDH phenotype (3A) is positively correlated with mean annual water temperature at each location. Based on my observations, I propose that clinal variation exists among the populations of Asterias forbesi that I have observed.

Certain specific populations of Asterias forbesi provide good examples of the influences of ecology and geography in evolutionary processes. For example, Merigomish Beach, Nova Scotia is ecologically similar to the Long-Island Sound-Cape Cod region. This ecological similarity is reflected in certain similarities of the animals from both areas. Specifically, animals from Merigomish and Long-Island Sound are somewhat alike in the ranges of weights of their carinal ossicles, and in frequencies of their XDH phenotypes. This evidence supports the hypothesis that geographically remote but ecologically similar environments favor selection for the same phenotypes. The salt pond at Blue Hill Falls, Maine is important in demonstrating the influence of geographic isolation on evolutionary processes. The salt pond maintains a population of animals with a high frequency (90%) of one of two XDH phenotypes, and ossicle weights almost identical to those of the congener of Asterias forbesi, the sea-star Asterias vulgaris. These conditions may be attributable to a combination of factors, including reduced gene flow, natural selection, founder effect, and possible hybridization.

Seasonal isozymal variation was investigated in Asterias forbesi collected at Pretty Marsh and the salt pond at Blue Hill Falls, Maine. No significant seasonal variation exists in the

frequencies of XDH phenotypes at either location. The two populations do differ significantly on the basis of XDH phenotypic frequencies. At Pretty Marsh, one MDH phenotype, designated 3A, shows temperature correlated seasonal variation. Esterase and phosphatase phenotypes show seasonal variation at both locations, and also differ between populations at certain times of the year. In some instances, seasonal variation in the frequencies of certain esterase and phosphatase phenotypes is directly attributable to the nutritional status of the animals and differences between populations of these phenotypic frequencies are related to the food available at each location.

INTRODUCTION

A. Reasons For Studying Asterias forbesi(Desor)

The common sea-star Asterias forbesi has been used in a variety of investigations which have made significant contributions to our understanding of asteroid biology. Some of the areas of investigation involving Asterias forbesi have included growth rates (Mead, 1900), natural history (Galtsoff and Loosanoff, 1939), echinoderm endocrinology (Chaet and McConnaughy, 1959), ecology (Ernst, 1967; Menge, 1979), biochemical genetics (Schopf and Murphy, 1973), and biogeography (Franz, Worley, and Merrill, 1981). With the exception of the paper by Schopf and Murphy (1973) no studies exist specifically concerned with the evolution or ecological genetics of Asterias forbesi. Because of its biogeographic distribution and its evolutionary relationship to its congener Asterias vulgaris Verrill, Asterias forbesi offers an opportunity to investigate processes of microevolution in the sea. Specifically, north of Cape Cod, populations of Asterias forbesi in the intertidal zone are restricted to certain localized areas, and are thus somewhat geographically isolated from one another (Schopf and Murphy, 1973). Asterias forbesi was chosen for this study because this situation represents an opportune natural laboratory for investigating the influence of geography and ecology on evolutionary processes which may effect intraspecific variation in this sea-star. Such evolutionary processes are gene flow, genetic drift, founder effect, and

natural selection (Mayr, 1975), and possible hybridization with the congener of Asterias forbesi, the sea-star Asterias vulgaris (Ernst, 1967; Sherman, 1971).

In summary, I have chosen to study Asterias forbesi primarily for the following reasons:

(1) The literature indicates that no studies exist concerning geographic variation in Asterias forbesi.

(2) The geographic distribution of Asterias forbesi offers an opportunity to study the effects of geographic isolation on intraspecific variation in this sea-star.

B. Taxonomy

Taxonomic characteristics of populations of Asterias forbesi and Asterias vulgaris on the eastern coast of the United States have been reviewed by Coe (1912), Aldrich (1956), and Walker (1973). These characteristics are summarized in Table 1. In addition, larval morphology (Agassiz, 1877) and the number of adambulacral plates (Clark, 1904) differ between these two sea-stars. Based on protein electrophoresis, Schopf and Murphy (1973) indicate that 67% of the genes of Asterias forbesi and Asterias vulgaris overlap; as a result, they assign these animals to semi-species. Franz (1981), on the other hand, using zoogeographic evidence, regards Asterias forbesi and Asterias vulgaris as being separate species. Of possible evolutionary significance on the United States east coast is the existence of poorly

TABLE 1

Characterization of *Asterias forbesi* and *Asterias vulgaris*

Characteristic	*A. vulgaris*	*A. forbesi*
Shape of ray	Thick at base Pointed at tip, i.e. strongly tapered	Slender at base Blunt at tip, i.e. with sides nearly parallel
Optical peduncle	Present	Absent
Ossicles	Carinal ossicles form a distinct line from disc to tip of ray on aboral surface	No distinct series of carinal ossicles
Major pedicellariae	Long, pointed	Broad, rounded
Color of madreporite	Pale yellow	Orange-red
Skeleton	Soft	Firm
Color	Rarely dark green	Rarely pink
Number of adambulacral plates	75, each side	60, each side

This table is modified from Coe (1912) and Aldrich (1956). Information concerning the tapering and coloration of the rays is based on observations made by Swan (1975). Walker (1973) has also considered coloration in these two species. Information about the number of adambulacral plates comes from Clark (1904), and is presently being reviewed by Maureen Downey of the Smithsonian Institute.

described, morphologically atypical specimens of Asterias forbesi reported primarily from the vicinity of Woods Hole and Long-Island Sound. Some evidence for the existence of these specimens is anecdotal and comes from numerous investigators who are familiar with the sea-stars found along the east coast; documentation has been provided by Clark (1904), Perlmutter and Nigrelli (1960), Ernst (1963), Sherman (1971), and Walker (1973). All these authors provide circumstantial evidence for the existence of hybrids. In addition, Perlmutter and Nigrelli (1960) and Ernst (1963) provide evidence for the existence of morphologically distinct subpopulations of Asterias forbesi, based on the length-width ratio of the rays. Ernst (1963) reports also on the occurrence of forbesi-like animals having major pedicellaria more like those of Asterias vulgaris and vulgaris-like animals having major pedicellaria more like those of Asterias forbesi. While these subpopulations are reported from the Long-Island Sound region, it is possible that they are indicative of the existence of other types of intraspecific variation in other populations. Since the nature and extent of intraspecific variation over the geographic range of Asterias forbesi has not been studied, I have undertaken this investigation of geographic and seasonal variability in this sea-star. Important in a study of this type is an appreciation of the potential for gene flow and the existence of possible agents of natural selection in geographically separated populations. Some information about the potential for gene exchange among geographically separated populations of Asterias forbesi, and possible selective forces acting on those populations can be gained from a consideration of the natural history and

ecology of Asterias forbesi.

C. Natural History of Asterias Forbesi

Aspects of the natural history of Asterias forbesi which are important to this study are those immediately concerned with fitness and the potential for gene flow. Observations relevant to these points were initially reported by Galtsoff and Loosanoff (1939).

Asterias forbesi is a dioecious, benthic, marine invertebrate possessing enormous reproductive potential. Adults do not have any directly observable external sexually dimorphic traits. Spawning occurs in June in Woods Hole and Long-Island Sound as water temperatures achieve 15^{o}C (Sherman, 1966). While no precise estimate of gamete production exist for Asterias forbesi, (Gemmill, 1914) records that a female Asterias rubens from the Firth of Clyde released 2,500,000 eggs during a spawning period lasting two hours. More recently, Walker (1980) estimates that male Asterias vulgaris from the New Hampshire coast are able to produce 160,000,000 spermatozoa per testis per year. It is reasonable to suppose that the numbers of gametes produced by Asterias forbesi are great and approximate those produced by the asteroids for which we have specific information. External fertilization and subsequent development result in a series of planktonic larval stages. The final larval stage, the brachiolariae, settle, and complete metamorphasis into adult sea-stars. When food is abundant, adult sea-stars may become sexually mature within about one year (Mead, 1900; Galtsoff and Loosanoff, 1939). The duration of the larval phase may be up to several months in length and is governed by

by environmental factors such as temperature and the availability of food. Given the number of gametes and larvae produced by Asterias forbesi, the potential for gene exchange among geographically separated populations would appear to be great. Also migration of adults could account for gene exchange, although Galtsoff and Loosanoff (1939) report that Asterias forbesi makes random movements of about a mile in one year. Chief among the factors which may influence reproductive isolation from Asterias vulgaris are food availability, and the possible existence of seasonal temperature differentials as they effect gamete production and spawning. Walker (1980) has suggested the possible existence of a temperature controlled mechanism involving hormone-like substances which trigger the evacuation of spermatozoa in Asterias vulgaris.

D. Geographical Distribution and Ecology

Populations of Asterias forbesi are found from Florida to the Canadian Maritime Provinces, and from the intertidal to depths of 100 meters. North of Cape Cod, this sea-star is restricted to warmer, inshore bays, and although it has been reported in the Gulf of St. Lawrence and St. Ann's Bay, Nova Scotia, it becomes increasingly rare as one proceeds north. It is replaced by populations of Asterias vulgaris, an animal which is found from Cape Hatteras to Labrador. South of Cape Cod, Asterias vulgaris is limited to deeper, colder waters of 650 meters. The two sea-stars are sympatric south of Cape Cod and in certain harbors north of the Cape (Schopf and Murphy, 1973; Walker, 1973).

The transect used in collecting specimens for this study, encompassed most of the range of Asterias forbesi noted above. This transect is unique in that it is approximately 1200 air miles in length, it encompasses about 20^{o} of northern latitude, and it involves Arctic, Boreal, Virginian, Carolinian, and Tropical provinces (Johnson, 1934; Cerame-Vivas and Gray, 1966). The extensive geographical range of collection sites permits considerable local and regional differences of ecologically significant factors such as sea-water temperature and the degree of physical isolation of populations from one another.

Asterias forbesi is more common and uniformly distributed intertidally in the Virginian and Carolinian provinces, with their Gulf Stream influence, substrates of unconsolidated material, low tidal amplitudes and gradient-like distributions of environmental factors such as temperature (Gosner, 1971). The Arctic and Boreal provinces are more physically heterogeneous than are the Virginian and Carolinian (Gosner, 1971; Menge, 1979). Asterias forbesi is less abundant and uniformly distributed in populations north of Cape Cod, which are influenced by low water temperature, high tidal amplitudes and a rocky, geologically incised coastline. While the latter creates a number of locally varying ecological conditions primarily in the Gulf of Maine, spatial and ecological heterogeneity also exist on a larger scale in the Boreal province. This is due to the existence of areas such as the Northumberland Strait which has ecological features somewhat similar to Long Island Sound (Bousfield and Thomas, 1975). Bousfield and Thomas (1975) report the existence of a number of species of intertidal

animals which display a discontinuous distribution. These are animals which prefer warm waters, and are found south of Cape Cod northward to the Gulf of Maine. The animals do not appear to be present in Gulf of Maine waters, but are found again in the warmer waters of the Gulf of St. Lawrence in such areas as Northumberland Strait. It may be that intertidal populations of Asterias forbesi are discontinuous in this manner.

Information concerning the nature of possible selective forces acting on sea-star populations is of importance to this study of geographic and seasonal variation in Asterias forbesi. Investigations of the natural history and ecology of Asterias forbesi by Coe (1912), Huntsman and Sparks (1924), Galtsoff and Loosanoff (1939), G. Smith (1940), Ernst (1963), and Menge (1979) indicate that a variety of potentially important selective forces may act on Asterias forbesi; among these are storms, food availability, larval mortality based on predation by fish, adult mortality based on predation by birds and lobsters, and possibly weak exploitation competition with its cogener Asterias vulgaris (Menge, 1979).

It is possible that temperature of the sea-water at each location is of particular importance as an instrument of natural selection acting on Asterias forbesi. The temperature of sea-water is considered to be important because of the role temperature has in the adoptive significance of isozymes (Koehn, 1969; Hochachka and Somero, 1973), and the function of temperature in regulating reproductive and feeding behavior in Asterias forbesi (MacKenzie, 1969). Mean annual sea-water temperatures estimated for each sampling location during the present study are shown in Figure 1.

FIGURE 1

Ranges in sea-water temperature at each sampling location. These ranges are based on information from U.S. Department of Commerce Coast and Geodetic Survey Special Publication No. 278(1951), Pyle (1962), Walford and Wickland (1968), Weiler and Keely (1980), and personal communications with Mr. Archie Carr of the Massachusetts Division of Marine Fisheries; Mr. Ken Drinkwater of the Bedford Oceanographic Institute, Nova Scotia, Canada; Mr. William Hettler of the National Marine Fisheries Laboratory at Beaufort, North Carolina; Mr. Fred Thurburg of the National Marine Fisheries Laboratory at Milford, Connecticut.

Temperature ranges at Blue Hill Falls and Pretty Marsh are based on observations by Towle (1980). The numbers beside each temperate range, designated with a numeral and the letter M indicate the length of the foraging season in months, at that particular location.

BF - Beaufort, North Carolina

LI - Long Island

CC - Cape Cod

PM - Pretty Marsh

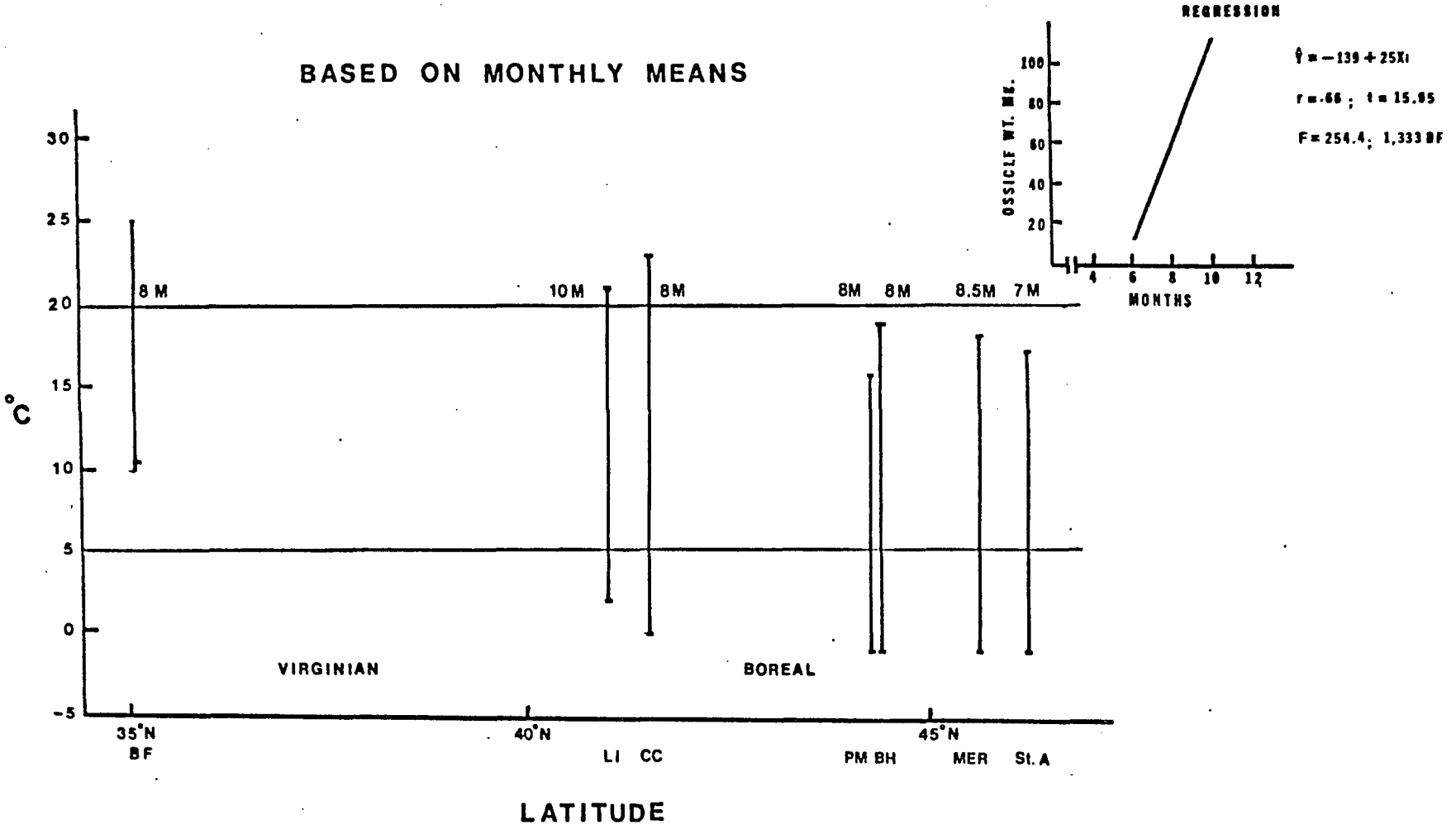
BH - Blue Hill Falls

MER - Merigomish

STA - St. Ann's Bay

SEA-WATER TEMPERATURE RANGES

BASED ON MONTHLY MEANS



While mean annual temperatures at each location are inversely correlated with latitude, the mean annual range in temperature at each location is related to particular geographic circumstances. Narrow ranges are found at Beaufort, North Carolina and Pretty Marsh, Maine. Wider ranges are found at Long Island Sound, Cape Cod, Blue Hill Falls, Maine, Merigomish, and St. Ann's Bay, Nova Scotia (Figure 1).

E. Geographic Variation

The eastern coast of the United States and areas in the southwestern Gulf of St. Lawrence, Canada, form a geographic assemblage that is unique in its physiographic, hydrographic, biotic, and climatic characteristics. Taken together, these features suggest that the eastern coast of the United States and the Gulf of St. Lawrence provide an opportune, natural laboratory for a study of microevolution involving Asterias forbesi.

Crucial to an understanding of the processes involved in microevolution is the estimation of geographically-related intraspecific variation. Studies concerned with intraspecific variation are of significance in elucidating the relative influence of gene exchange versus natural selection on a population of organisms. Moreover, the consideration of intraspecific variation is helpful in determining variation attributable to possible hybridization, and in assessing physiological adaptation of individuals of populations.

The existence of physiological races, or ecotypes within the Echinodermata has not been studied extensively with electrophoretic

techniques. Rutherford (1977) investigated geographical variation in morphological and electrophoretic characters in the holothurian, Cucumaria curata from the western coast of the United States. Using five distinct populations he found no polymorphism for any of the eight enyzmes that he observed. Furthermore, he recognized no clinal relationship between geographical location or habitat and any of the morphological characters he examined. His morphological data suggested that random differentiation has occurred among the populations that he studied. Whether or not random differentiation was attributed to genetic drift was not stated. Using protein variation resulting from electrophoretic analysis, Marcus (1977) examined genetic variation in five populations of the sea-urchin Arbacia punctulata. Of the twelve enzyme systems she assayed, five showed evidence of clinal variation in four populations distributed over a transect from Woods Hole, Massachusetts to Beaufort, North Carolina.

F. The Problem

Until the present study, no extensive examination of morphological and protein variation among geographically separated populations of Asterias forbesi has been performed. The existence of morphologically distinct sub-populations of Asterias forbesi described by Perlmutter and Nigrelli (1960) and Ernst (1967) raises the question whether or not any exchange of genetic information is occurring among geographically distant populations of Asterias forbesi. Genetic exchange might result from the migration of adults and by the wide dispersal of planktonic larvae.

If variation does exist among geographically distant populations of Asterias forbesi, it may reflect basic differences generated in situ by unique selective forces in local environments. It is possible that variation among populations is attributable to genetic drift and founder effect (Mayr, 1963; 1975). Alternately, variation may result from a genetically based, phenotypically plastic response of populations to differing environmental conditions (Bradshaw, 1965; Ebert, 1980). Examples of variation which might be expected throughout the range of Asterias forbesi are clines, hybrid zones, and peripheral isolates (Mayr, 1975). Cline may represent the result of the interactions of gene exchange and natural selection, while peripheral isolates represent areas where selection is possibly the dominant influence (Mayr, 1975). A peripheral isolate located adjacent to a cline, then, should offer the opportunity to study the relative influences of selection and gene flow (Mayr, 1975).

The purpose of the present study was to determine whether or not variation exists among geographically separated or isolated populations of Asterias forbesi as demonstrated by an examination of protein and morphological characters. I further sought to relate detected patterns of variation to environmental factors such as sea-water temperature and possible routes of larval transport. This research included a comparison of protein variation in specimens collected throughout the year from an isolated salt pond at Blue Hill Falls, Maine, with specimens from Pretty Marsh, Maine. The latter location is about six miles away from the salt pond, but situated at approximately the same latitude. The sampling

location at Pretty Marsh is less isolated than the salt pond from Gulf of Maine influence. The investigation of seasonal variation was undertaken for the following reasons:

(1) Seasonally related isozyme variation has not been studied in Asterias forbesi.

(2) Knowledge of seasonally related isozymal variation may allow the detection of environmentally induced variation.

(3) Such studies are a meaningful way of comparing two populations of organisms inhabiting different habitats.

Finally, I attempted to explain detected seasonal variation in certain isozyme systems, in terms of the possible nutritional status of the animals. This was done in a laboratory experiment whereby both fed and fasted animals were compared on the basis of certain enzyme banding patterns.

MATERIALS AND METHODS

A. Collection and Maintenance of Sea-Stars

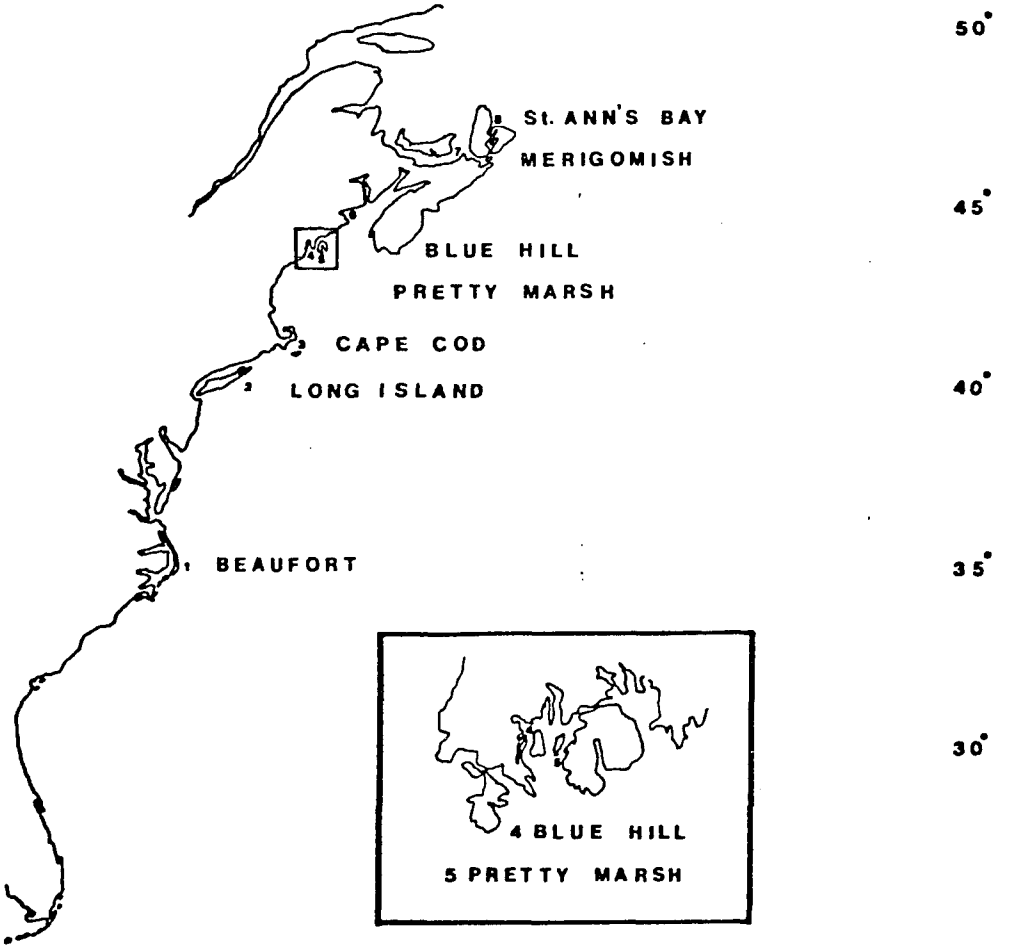
(1) Collections From Geographically Separated Populations.

In July and August, 1979 specimens of *Asterias forbesi* ranging in size from R = 23 mm to R = 110 mm, were collected from each of the following locations: Beaufort, North Carolina; Long Island Sound at Milford, Connecticut; the Cape Cod Canal at Bourne, Massachusetts; Blue Hill Falls, Maine; Pretty Marsh, Maine; Merigomish Beach, Nova Scotia; and St. Ann's Bay, Cape Breton, Nova Scotia (Figure 2). Specimens from Beaufort, North Carolina were obtained by dredging, by members of the supply department at the Marine Laboratory of Duke University. Specimens from Long Island Sound were collected by mopping off-shore from the National Marine Fisheries Laboratory at Meriden, Connecticut. These animals were collected by John Volk of Long Island Oyster Farms and by Dr. Fred Thurburg of the National Marine Fisheries Laboratory. Animals were obtained at the other locations by SCUBA from the intertidal and shallow subtidal zones. The specific collection procedure at these locations involved swimming along transects about 50 meters long, roughly parallel to the shoreline, and at intervals of about 10 feet, diving to the bottom and collecting every *Asterias forbesi* within an arm's radius. Animals too small to be readily identified, or too small to provide amounts of caecal tissue adequate

FIGURE 2

Collection sites along the eastern United States Coast and Canada from which specimens of *Asterias forbesi* were obtained.

COLLECTION SITES



for electrophoretic analysis were not collected. Fifty animals were obtained from all locations except Beaufort, North Carolina. Thirty-five animals were obtained from this location. In all cases, animals obtained were held overnight in a circulating sea water system maintained at a salinity of 32 $^{o}/oo$ and a temperature of 13^{o}C. Within a day after the animals were brought to Durham, New Hampshire, portions of caecal tissue from each animal were removed and frozen, at -90^{o}C pending electrophoretic examination. The remainder of each animal was preserved in 70% ethyl alcohol.

(2) Collections For The Determination Of Seasonal Isozymal Variation.

Seasonal variation in isozymal patterns was assessed using samples of pyloric caecal tissues obtained from animals collected from an enclosed salt pond at Blue Hill Falls, Maine, and a location near the town dock at Pretty Marsh, Maine. Early in March, May, August, and October of 1980, twenty-five specimens of Asterias forbesi were collected by SCUBA as noted previously, from intertidal to shallow subtidal zones at each location. After each field collection, animals were returned to the laboratory and held overnight in a circulating sea-water system maintained at a salinity of 32 $^{o}/oo$ and a temperature of 13^{o}C. Within a day after collection, portions of pyloric caecal tissue were removed from each animal and frozen at -90^{o}C. The remainder of each animal was preserved in 70% ethyl alcohol.

B. Preparation of Tissues

(1) Analysis Of Isozymes.

The method of preparation of tissues was identical for studies of geographical and seasonal variation in isozymes. The tip of ray was removed and a mid-dorsal incision was made along its length. With large animals ($\geq$ about 5 cm in diameter, R = 25 mm) an entire caecum was removed, rinsed in cold homogenizing buffer at $2^{o}C$ - $3^{o}C$ and blotted dry. From the mid-point of the caecum, portions of tissue weighing 0.1 gram were cut out with a razor blade and placed in a 1.2 ml Cryovial (A.S. Nunc, USA) containing 0.15 ml of cold ($2^{o}C$ - $3^{o}C$) homogenizing buffer. In order to obtain 0.1 gram of tissue from small animals ($\leq$ about 5 cm in diameter) occasionally an entire organ or both organs from a given ray were used. Otherwise, the preparation of tissues was the same as for larger animals. In both investigations, three replicates of tissue were taken from the pyloric caeca of each animal. Two were placed in buffer system I, one in buffer system II (Appendix I). When electrophoresis was performed, samples were thawed over a 5-minute period at room temperature, placed immediately in a slurry of ice and water ($2^{o}C$ - $3^{o}C$), and homogenized in the Cryovials with a conical tipped, teflon tissue grinder. Homogenates were centrifuged in the Cryovials at 24,150 xg at $0^{o}C$ for 10 minutes using a Sorvall RC 2-B refrigerated centrifuge equipped with an SM-24 rotor.

(2) Preparation Of Carinal Ossicles.

Ray was removed from all animals collected at each sampling location and the viscera were removed. A block of the body wall (1 cm x 2 cm) 1.0 centimeter from the proximal end of the ray was excised from the mid-dorsal surface. In order to separate overlying

tissues from the skeletel elements, this portion of the body wall was placed in a test tube containing 4 ml of 10% KOH for 10-12 hours. Dissolved material was removed with a Pasteur pipette, and the ossicles were washed with distilled water until clean. Each group of resulting ossicles was then filtered on pre-weighed 7 cm Whatman #1 filter paper. Filtered ossicles were dryed at room temperature and weighed.

C. Preparation Of Polyacrylamide Gels For Electrophoresis

Separations of isozymes were accomplished by gel electrophoresis using a pair of vertical slab chambers described by Roberts and Jones (1972) and manufactured by Aardvark Instruments, Lombard, Illinois. Samples examined for isozymal variation were separated in 3 mm thick, 6% polyacrylamide gels (Cyanogum-41, Sigma). Samples examined for seasonal isozymal variation were separated in gels that were similar in all ways to those noted above, except thickness; 6 mm gels were used and then subsequently sliced horizontally to give two 3 mm thick gels. This procedure permitted the detection of two different enzyme systems for each electrophoretic run. Despite differences in the thickness of the gels, preparation was the same for both studies. In both cases, gels were poured about 8 hours before use. The electrophoretic chambers were filled with buffer (either system I or II, depending on which enzyme system was being examined) covered and refrigerated overnight at $4^{o}C$. Gels were prepared by a dilution of a 30% Cyanogum-41 stock solution (Sigma) of either buffer system I or II as reported by Hubby and Lewontin

(1966) and Lewontin (1975). Polymerization of acrylamide was catalyzed with .02% tetramethyethylenediamine (TMED; Sigma) and 10% ammonium persulfate (Sigma). The formula for gel preparation is given in Appendix I.

D. Methods Of Electrophoresis

Electrophoretic procedures were identical in studies of geographically related and seasonally related isozymal variation. The electrophoretic chambers containing prepared gels and buffer were attached to a Neslab circulating bath cooled to about $4^{o}C$ with a Neslab PBC-Z Bath Cooler. Cold 20% ethylene glycol was circulated continually through the boxes until the bath temperature stabilized at $-5^{o}C$. Gels were maintained at this temperature for at least one-half hour prior to each electrophoretic run. Gels were cast with a slot former which provided 24 pockets so that homogenates from different individuals could be separated simultaneously. Representatives from each geographic location were run in the same gel. 10 ul of homogenate from a single Asterias forbesi was added to each pocket; the end pockets each received 5 ul of .01% Bromphenol blue dissolved in the appropriate homogenizing buffer as a tracking standard. Electrophoresis for the majority of enzyme systems was carried out at 90 mA and 350 V until the dye marker reached a point 5.5 cm from the origin. The time required for the dye marker to travel 5.5 cm was about 1½ hours. Leucine aminopeptidase (LAP) required a longer separation period, so the dye marker was allowed to migrate to a point 10.5 cm from the origin. The time required for the dye marker to travel this distance was about 3 hours.

At the conclusion of a run, enzyme activity was visualized by substrate specific staining at room temperature (25°C). Subsequently, the gels were washed in distilled water and fixed in a solution of methanol, water, and glacial acetic acid (5:5:1, by volume). The gels were photographed and band patterns reproduced on graph paper by hand.

E. Methods Of Enzyme Detection

(1) Geographically-related Isozyme Patterns.

Each homogenate was assayed for both xanthine dehydrogenase (XDH) and malate dehydrogenase (MDH) activity using a modification of the protocols of Hubby and Lewontin (1966) and Lewontin (1975). XDH was stained at pH 7.5 using .05M hypoxanthine as a substrate; MDH was stained at pH 8.5, with 0.1M DL malic acid as a substrate (Table 2).

(2) Seasonally-related Isozyme Patterns.

Homogenates of caecal tissues obtained from specimens collected at Blue Hill Falls and Pretty Marsh were examined seasonally in attempting to detect variation in seven enzyme systems. These enzyme systems are: xanthine dehydrogenase (XDH), malate dehydrogenase (MDH), glutamate oxaloacetate transaminase (GOT), non-specific esterases (EST), acid phosphatase (AcP), alkaline phosphatase (AlkP), and leucine amino peptidase (LAP). Tissues used to detect these enzyme systems were run in 6 mm thick gels (except LAP), which were sliced horizontally; each slice was then stained for a different enzyme system (Table 2). Most staining procedures were modified from Hubby and Lewontin (1969) and

TABLE 2

Staining Conditions Utilized In Detecting Isoenzymes Assayed For Geographic And Seasonal Variation

Enzyme System	pH	Substrate	Stain
XDH	7.5	.05M Hypoxanthine	Nitroblue tetrozolium
MDH	8.5	.1M DL Malic acid	Nitroblue tetrozolium
GOT	8.0	α-Ketoglutaric acid	Fast violet B salt
LAP	5.2	L-leucyl-β-Naphthylamide-HCL	Fast black K
AcP	5.0	Na-α-Naphthyl phosphate	Fast blue BB
AlkP	9.2	Na-α-Naphthyl phosphate	Fast blue BB
EST	6.5	α-Naphthyl acetate	Fast red TRN

Lewontin (1975). Procedures for GOT are modified from Shaw and Prasad (1969). Detailed procedures for enzyme detection are shown in Appendix I.

F. Methods Used In Detecting The Effects of Feeding And Fasting On Isozyme Banding Patterns.

In June of 1980, 20 Asterias forbesi were collected by SCUBA from both the salt pond at Blue Hill Falls and Pretty Marsh, Maine, using collection techniques noted earlier. Upon returning to the laboratory at Durham, New Hampshire, the animals were placed in individual containers in a circulating sea-water system held at a temperature of $13^{o}C$ and a salinity of $32^{o}/oo$. Prior to the experiment proper, the animals were allowed to acclimate to these conditions for several days. The animals from each respective population were divided into two treatment groups; that is, a "fasted" group of ten animals, and a "fed" group of ten animals. Fed animals received and consumed a single, opened mussel 2 inches long, each day during the two week trial period. Animals in the fasted group received a single, opened mussel half-way through the trial period. At the end of the two-week period, surviving animals were sacrificed and portions of caecal tissue removed and frozen at $-90^{o}C$ pending electrophoretic analysis of isozymes. Preparation of tissues, gel preparation, electrophoresis, and staining techniques were the same as those previously described. Enzyme systems examined for the effects of feeding or fasting were: xanthine dehydrogenase, cytoplasmic malate dehydrogenase, esterases, acid and alkaline phosphatases.

RESULTS

A. Variation Between Geographically Separated Populations

(1) Morphological Variation In Skeletal Mass.

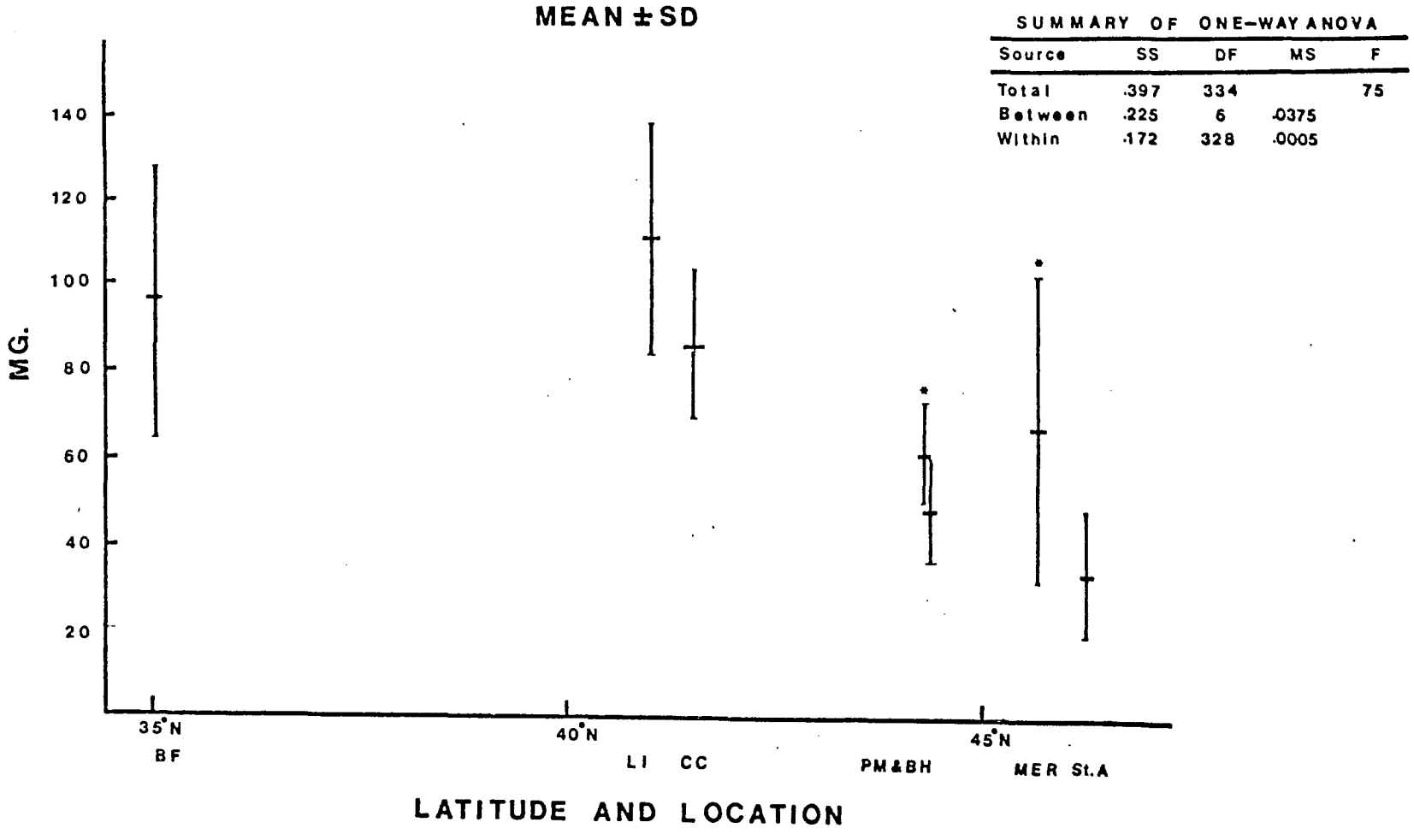
(Figures 3, 4, 5; Table 3)

Observations on the mean weight of carinal ossicles of the specimens from the various collection sites used in the present study demonstrate the existence of geographically related variation (Figure 3). Animals obtained south of Cape Cod tend to have heavier, more robust skeletons, while animals from locations north of Cape Cod tend to have delicate, reticulate skeletons (Figure 4). For seven populations examined, a one-way analysis of variance (Minitab II) based on 335 measurements indicates significant differentiation among the mean weights of carinal ossicles. Furthermore, a Student-Newman-Keuls pair-wise comparison test (Zar, 1974) shows that all populations differ significantly from each other except those at Pretty Marsh and Merigomish Beach (Table 3). The slight but significant negative correlation with latitude, $r = -.55$ (Figure 5) suggests the existence of clinal variation in carinal ossicle weights. Also, based on the latitude at which Blue Hill Falls is located, the mean carinal ossicle weight of resident animals is unusually low. Weights of carinal ossicles from animals collected at Blue Hill Falls would best fit the proposed cline at a more northern latitude; that is, between Merigomish Beach and St. Ann's Bay. If regression is re-run after assigning Blue Hill Falls a latitude of about 46^{o} N (midway between Merigomish

FIGURE 3

Mean weights (in mg) of carinal ossicles plotted against geographic distribution of specimens of Asterias forbesi utilized. The asterisks point out populations which do not differ significantly on the basis of Student-Newman-Keuls comparisons.

CARINAL OSSICLE WEIGHTS



SUMMARY OF ONE-WAY ANOVA

Source	SS	DF	MS	F
Total	.397	334		75
Between	.225	6	.0375	
Within	.172	328	.0005	

FIGURE IV

Rays of Asterias forbesi after treatment with 10% KOH. The animals were collected at Beaufort, North Carolina, Cape Cod, Massachusetts, and the salt pond at Blue Hill Falls, Maine.

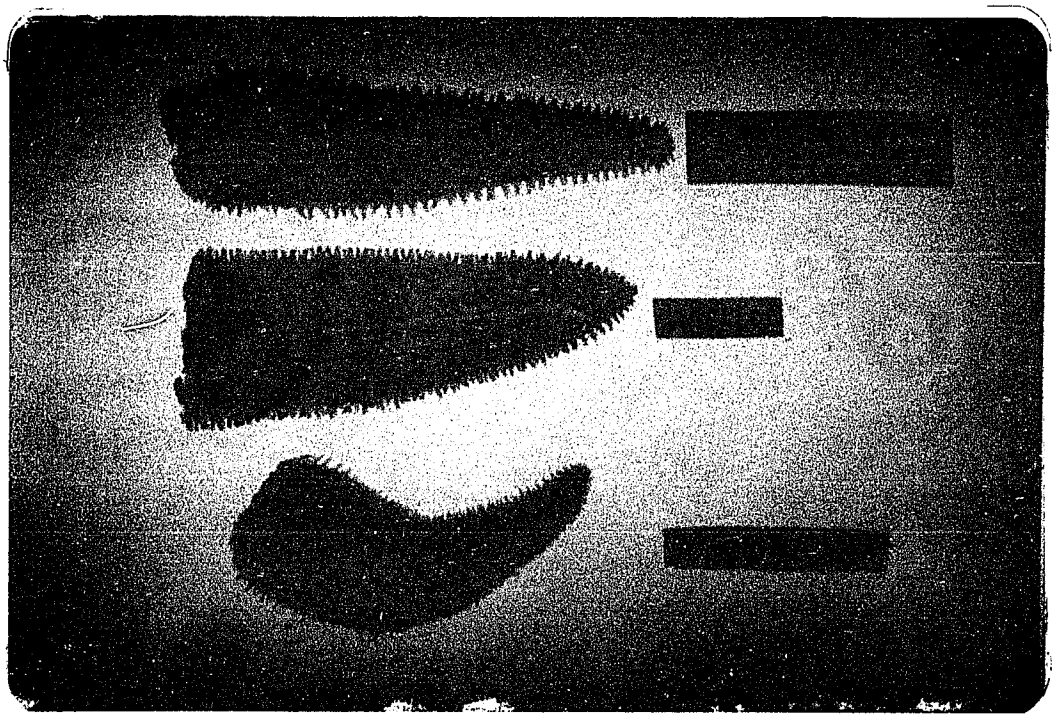


TABLE 3

Student-Newman-Keuls Pairwise Comparison Of The Mean Weights Of Carinal Ossicles

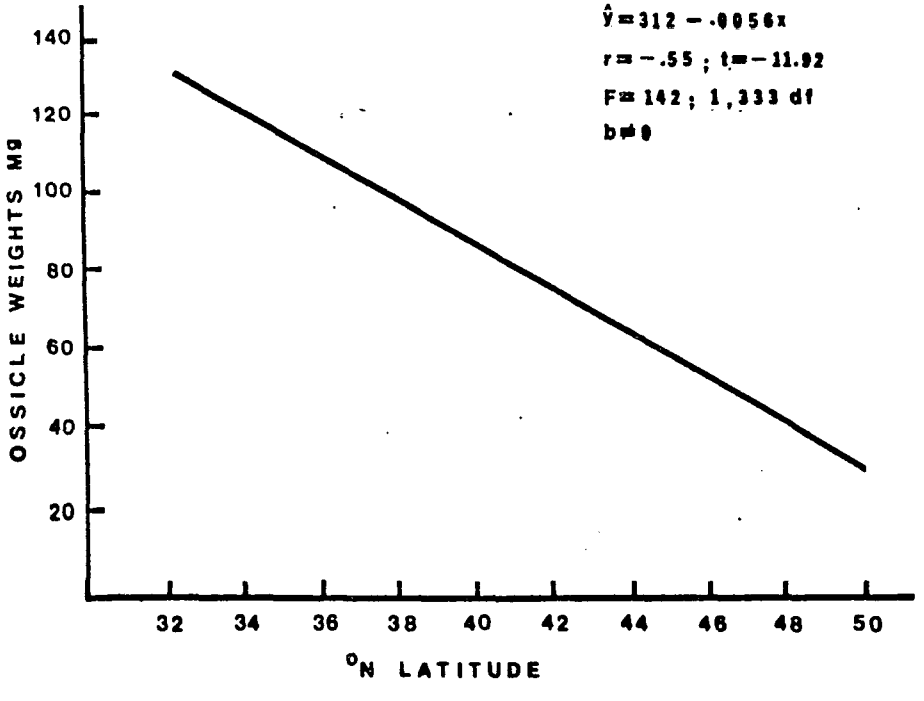
Comparison	q	Critical q	Conclusion Decision	H_o
Long Island-St. Ann's	24.68	4.29	Reject	u LI = u St. A
Lons Island-Blue Hill	19.94	4.17	Reject	u LI = u BH
Lons Island-Pretty Marsh	15.82	4.03	Reject	u LI = u PM
Long Island-Merigomish	13.92	3.86	Reject	u LI = u MER
Long Island-Cape Cod	7.91	3.31	Reject	u LI = u CC
Long Island-Beaufort	4.29	2.77	Reject	u LI = u BF
Beaufort-St. Ann's	18.0	4.17	Reject	u BF = u St. A
Beaufort-Blue Hill	13.7	4.03	Reject	u BF = u BH
Beaufort-Pretty Marsh	10.0	3.86	Reject	u BF = u PM
Beaufort-Merigomish	8.3	3.63	Reject	u BF = u MER
Beaufort-Cape Cod	2.86	2.77	Reject	u BF = u CC
Cape Cod-St. Ann's	16.77	4.03	Reject	u CC = u St. A
Cape Cod-Blue Hill	12.03	3.86	Reject	u CC = u BH
Cape Cod-Pretty Marsh	7.9	3.63	Reject	u CC = u PM
Cape Cod-Merigomish	6.0	3.3	Reject	u CC = u MER
Merigomish-St. Ann's	10.76	3.63	Reject	u MER = u St. A
Merigomish-Blue Hill	6.01	3.3	Reject	u MER = u BH
Merigomish-Pretty Marsh	1.9	2.77	Accept	u MER = u PM
Pretty Marsh-St. Ann's	8.9	3.3	Reject	u PM = u St. A
Pretty Marsh-Blue Hill	4.1	2.77	Reject	u PM = u BH
Blue Hill-St. Ann's	4.75	2.77	Reject	u BH = u St. A

FIGURE 5

A linear regression of the weights of carinal ossicles against latitude. Ossicular weights show a slight but significant negative correlation with latitude.

LINEAR REGRESSION

CARINAL OSSICLE WEIGHTS vs. LATITUDE



and St. Ann's Bay) then correlation with latitude becomes stronger (r = -.57). Furthermore, a comparison of the weights of carinal ossicles derived from specimens of Asterias forbesi from Blue Hill Falls with those of Asterias vulgaris from the same location, shows no significant differences. Specimens of both Asterias forbesi and Asterias vulgaris from the salt pond at Blue Hill Falls have strikingly similar skeletons in terms of the degree of calcification of the carinal ossicles; skeletons of both are light and reticulate.

(2) Biochemical Variation In Xanthine Dehydrogenase.

(Figures 6, 7, 8; Table 4)

Electrophoretic examination of the samples of the pyloric caeca of specimens from each of the seven locations demonstrates the existence of two XDH phenotypes. One phenotype, found in certain individuals, is comprised of a single band with an Rf of .36. The second XDH phenotype is found in the caecal tissues of other individuals and is made up of two bands: one band has an Rf of .36, the other an Rf of .40 (Figures 6 and 7). I tentatively interpret these bands as representing two monomorphic genetic loci. Both phenotypes are represented in all populations, but they occur in different proportions depending on geographic location (Figure 8, Table 4). The proportions of the two enzyme phenotypes vary significantly with geographic location (Figure 8). The slower moving band (.36) is more frequent in the caecal tissues of animals from Beaufort, North Carolina, while the faster moving band, Rf .40, gradually increases in frequency in animals of populations from more northern locations. A maximum frequency for the faster moving band is seen in the animals at Blue Hill Falls. Animals from Merigomish Beach and St. Ann's Bay show a decrease in frequency of this fast moving band.

FIGURE 6

Xanthine dehydrogenase phenotypes observed at each sampling location. Some individuals were characterized by the single, .36 band. Others were characterized by the two banded .36-.40 phenotype.

XDH PHENOTYPES

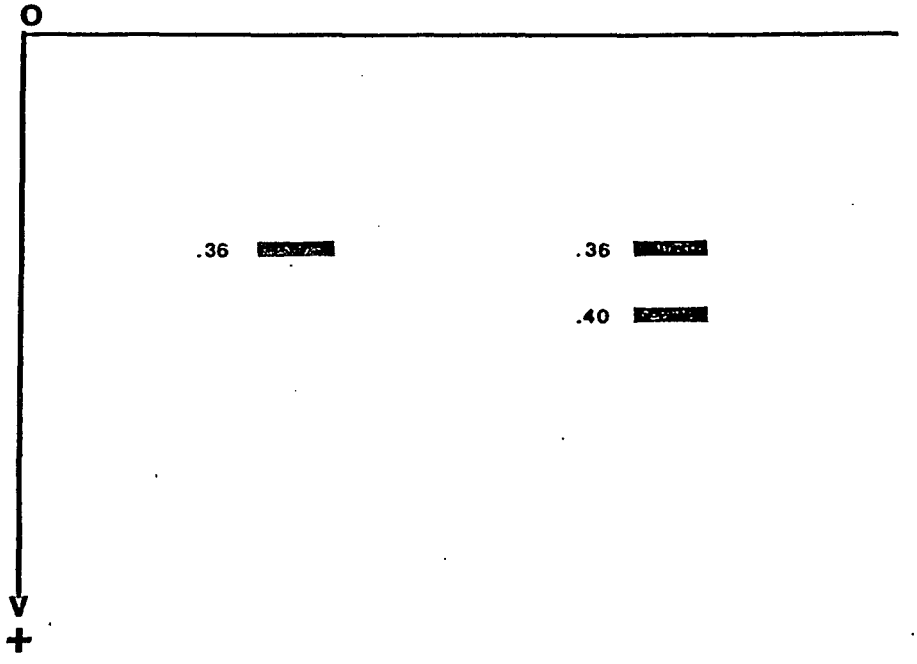


FIGURE 7

A photograph of a polyacrylamide gel showing xanthine dehydrogenase phenotypes observed at each sampling location.

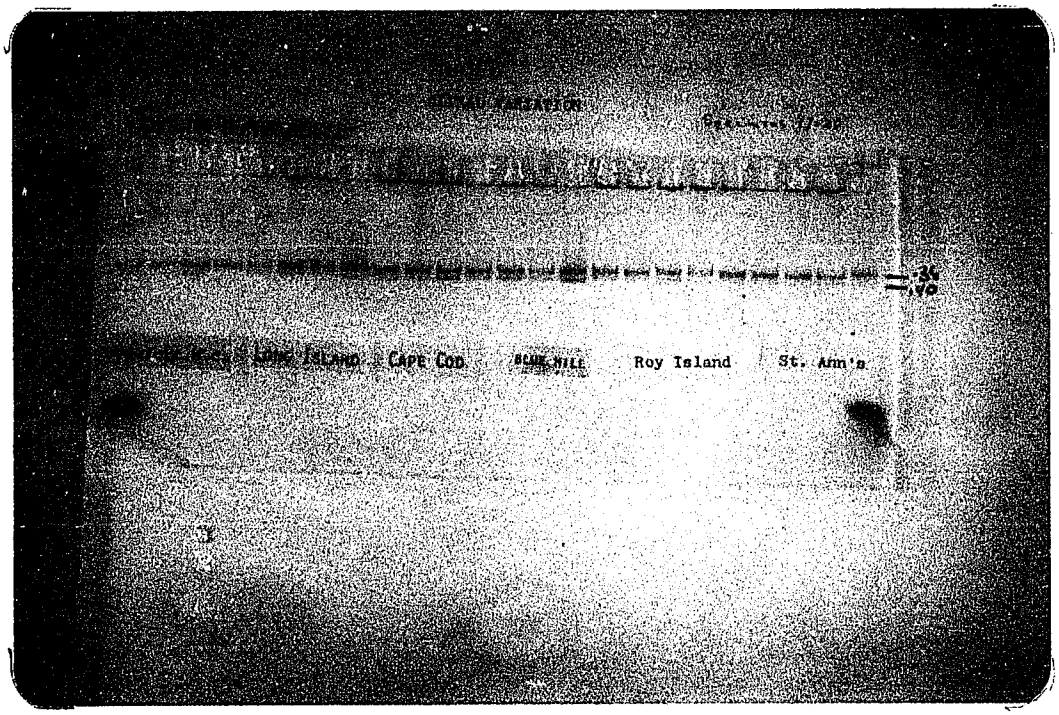

Cape Cod
Roy Island
St. Ann's

FIGURE 8

The relative frequencies of xanthine dehydrogenase phenotypes recorded at each sampling location.

XDH

PHENOTYPIC FREQUENCIES

HETEROGENEITY CHI SQUARE

H_o: ALL SAMPLES CAME FROM THE SAME POPULATION

X^2	DF	P
48.73	6	<.001

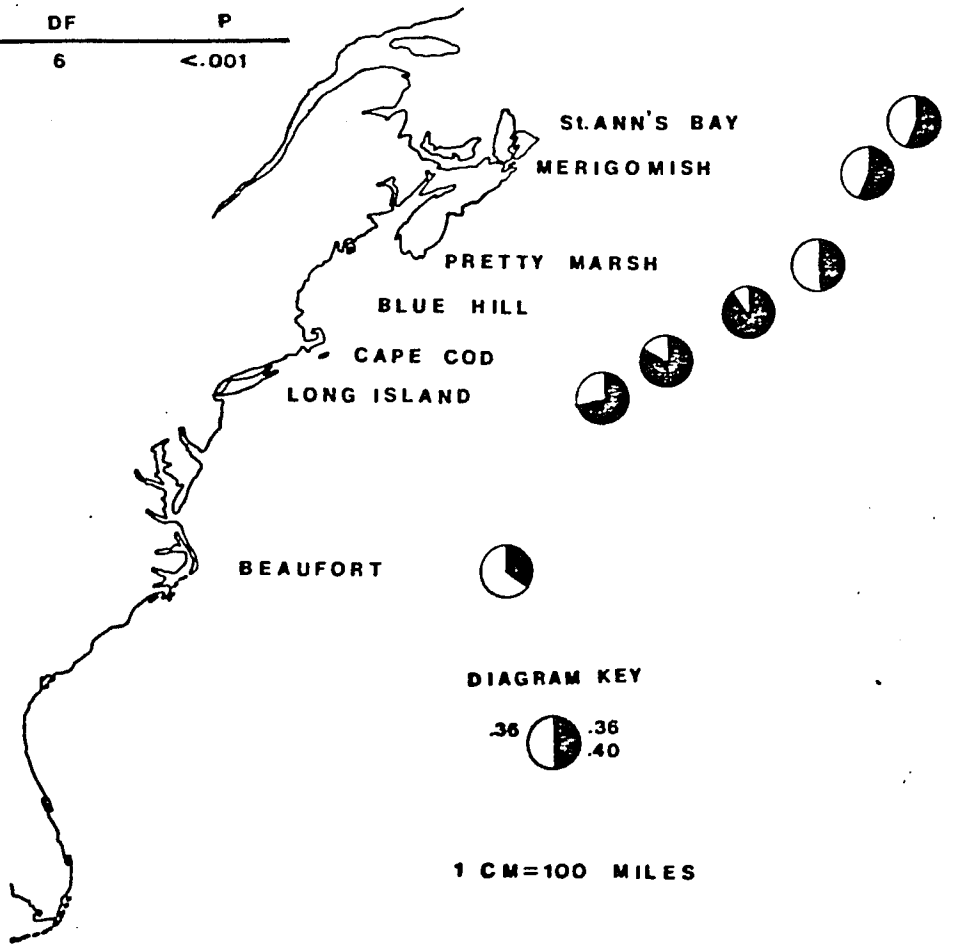


TABLE 4

The Relative Proportions Of Two XDH Phenotypes At Each Sampling Location

Location	N	Phenotype .36	Phenotype .36-.40
Beaufort	30	.64	.36
Long Island	56	.29	.71
Cape Cod	50	.18	.82
Blue Hill	50	.12	.88
Pretty Marsh	46	.60	.40
Merigomish Beach	50	.44	.56
St. Ann's Bay	50	.44	.56

Contingency chi-square analysis suggests that significant differentiation has occurred among populations for these phenotypes. Clinal variation is manifested by a progressive increase in the frequency of the fast moving band, and is most evident between Beaufort, North Carolina and Blue Hill Falls, Maine (Figure 8 and Table 4).

(3) Biochemical Variation In Malate Dehydrogenase.

(Figures 9, 10, 11; Table 5)

Electrophoretic examination of samples of the pyloric caeca of specimens from each of the seven locations demonstrates the existence of seven MDH phenotypes (Figures 9 and 10). Each phenotype possesses two darkly staining bands having Rf's of .27 and .29 respectively. The phenotypes are differentiated from one another by the possession of one or more of four additional, lighter staining bands of greater mobility. These bands have Rf's of .36, .38, .42 and .48 respectively. The phenotypes designated in Figure 9 as 2 and 3A are the most frequent of all of the phenotypes and are found in differing proportions at all seven sampling locations. Of the seven phenotypes, the 2 and 3A alone are found exclusively at the extremes of the collection range, that is, at Beaufort, North Carolina and St. Ann's Bay. The remaining phenotypes, designated in Figure 9 as 3B, 3C, 3D, 4A and 4B, are not found at Beaufort and St. Ann's Bay. Their occurrence is restricted to Long Island, Cape Cod, Pretty Marsh, and Blue Hill Falls. Individually these phenotypes are found in low frequencies compared to the frequencies of the 2 and 3A phenotypes (Table 5). Contingency chi-square analysis was performed on data for the 2 and 3A phenotypes only. The reason for this is

FIGURE 9

Cytoplasmic malate dehydrogenase phenotypes observed during the study of geographic variation.

MDH PHENOTYPES

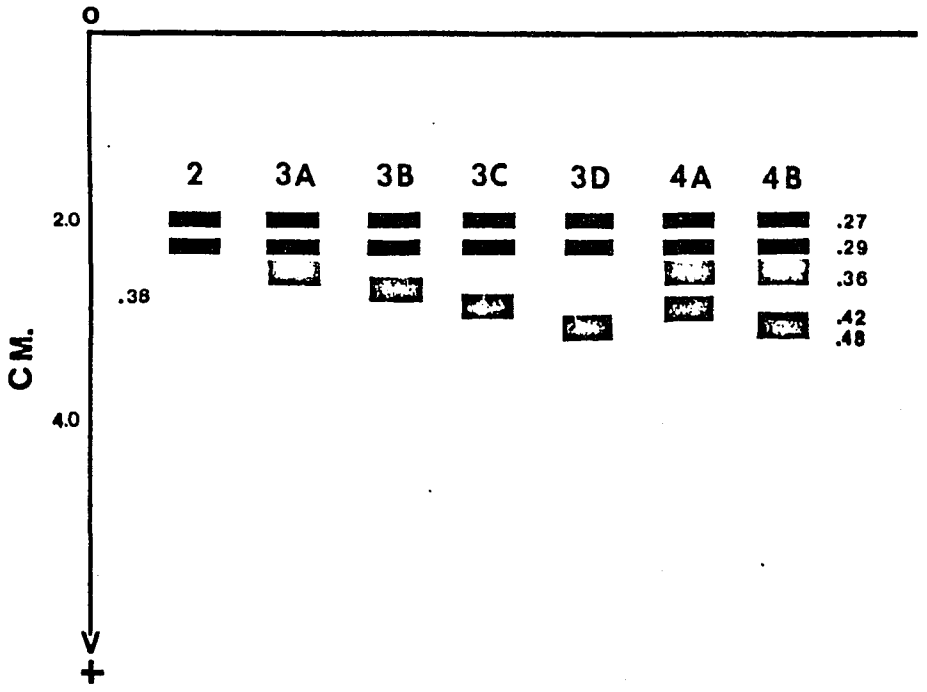


FIGURE 10

A photograph of a polyacrylamide gel showing cytoplasmic malate dehydrogenase phenotypes.

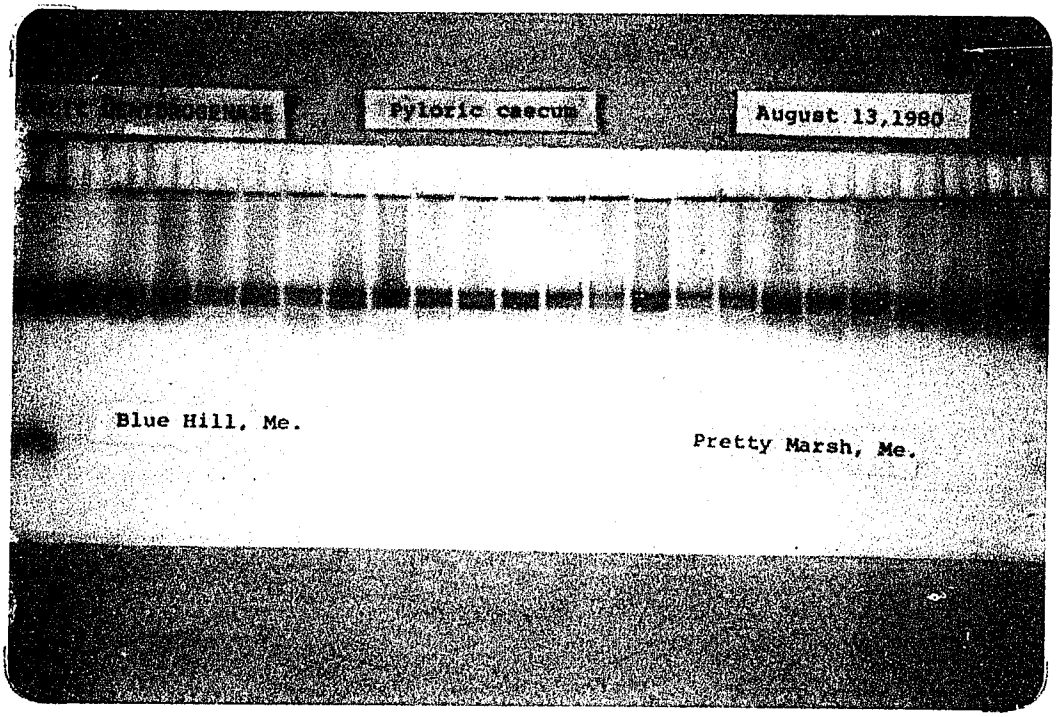

Pyloric caecum
August 13,1980
Blue Hill, Me.
Pretty Marsh, Me.

TABLE 5

The Relative Proportions Of Seven MDH Phenotypes At Each Sampling Location

		Phenotypes						
Location	N	2	3A	3B	3C	3D	4A	4D
Beaufort	30	.33	.67	0	0	0	0	0
Long Island	56	.33	.40	.036	.07	0	.11	.06
Cape Cod	50	.36	.32	.04	.12	.16	0	0
Blue Hill	50	.52	.30	.10	0	0	.06	.02
Pretty Marsh	46	.42	.46	.06	0	0	.02	.04
Merigomish Beach	50	.42	.32	.12	.04	.04	.04	.04
St. Ann's Bay	50	.74	.22	0	.02	0	0	.02

that the individual frequencies of the remaining phenotypes were low and resulted in an intolerable number of expected values less than 5, violating the 20% rule noted by Zar (1974). This rule states that only 20% of the cells in a contingency chi-square table may have expected values less than five. The 2 and 3A phenotypes were chosen for analysis because they were individually the most frequent at any location, they were both present in all populations sampled, and they both showed evidence of possible clinal variation. The chi-square analysis indicates that the relative frequencies of the 2 and 3A phenotypes differ significantly with respect to geographic location. Moreover, clinal variation is suggested in the 3A phenotype by the progressive decrease in the relative frequency of this phenotype from Beaufort, North Carolina to St. Ann's Bay. The frequency of the 3A phenotype is highest at Beaufort, decreases progressively to Blue Hill Falls, increases somewhat at Pretty Marsh, decreases again at Merigomish, and reaches its lowest frequency at St. Ann's Bay (Figure 11). The frequencies of the 3A phenotype at the various sampling locations correlates negatively with latitude ($r = -.86$), and positively with mean annual seawater temperature at each location ($r = .84$). Some evidence exists for clinal variation in the 2 phenotype. The frequency of this phenotype is lowest at Beaufort, North Carolina, increases progressively to Blue Hill Falls, Maine, shows a decrease at Pretty Marsh, increases again at Merigomish, and reaches its highest frequency at St. Ann's Bay. The 2 phenotype does not show a significant correlation with either latitude or mean annual seawater temperature.

FIGURE 11

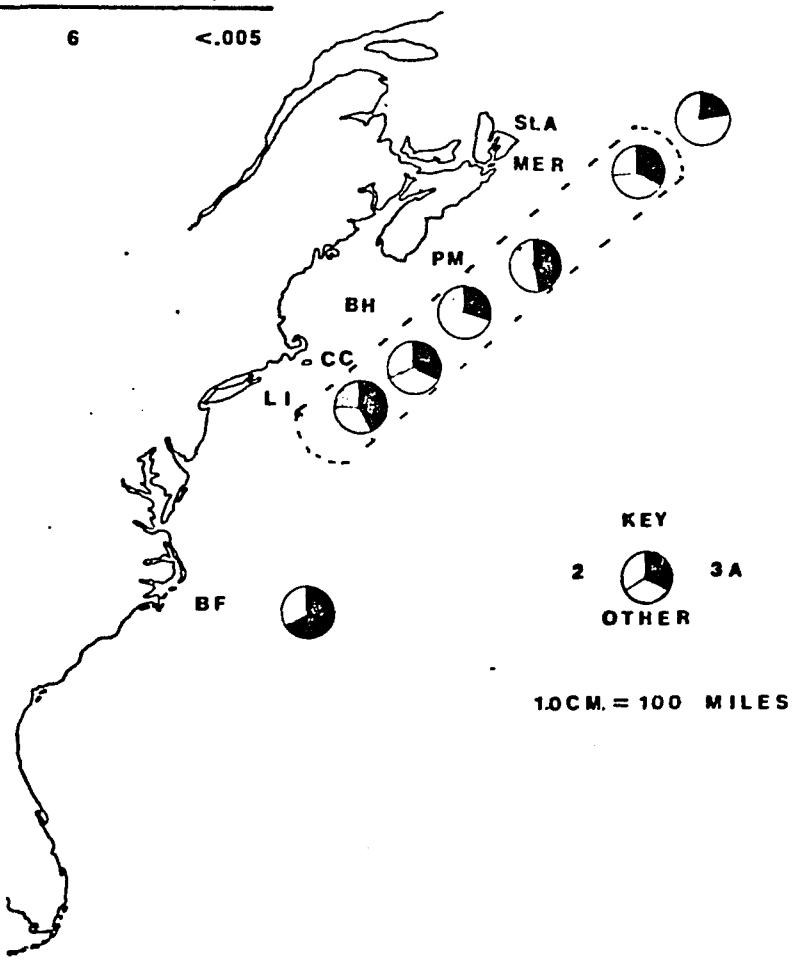
The relative frequencies of cytoplasmic malate dehydrogenase phenotypes observed at each sampling location.

MDH

PHENOTYPIC FREQUENCIES

HETEROGENEITY CHI SQUARE

X^2	DF	P
19.46	6	<.005



B. Seasonal Isozyme Variation At Blue Hill Falls And Pretty Marsh

(1) Seasonal Variation In Xanthine Dehydrogenase.

(Figure 12; Tables 6, 7, and 8)

Electrophoretic examination of caecal tissues of specimens collected from the salt pond at Blue Hill Falls during March, May, August, and October of 1980, reveals the existence of the same two XDH phenotypes which were reported earlier (section A2 of Results). The frequency of the .36-.40 phenotype remains high during all four seasons (Figure 12). Contingency chi-square analysis indicates no differences among seasons on the basis of the frequencies of the two XDH phenotypes (Table 6). No significant correlation exists with seasonal change in the temperatures of seawater. The same XDH phenotypes were detected at Pretty Marsh as were found at Blue Hill Falls, and as were reported earlier (Section A2 of Results). The frequency of the .36-.40 phenotype (Figure 12) remained low in March, May, and October but, rose slightly in August. Despite this slight rise in frequency, a contingency chi-square analysis indicates no significant difference among seasons, based on XDH phenotypic frequencies (Table 7). No significant correlation can be found with seasonal change in seawater temperatures (Figure 12). Contingency chi-square analysis indicates significant differences between populations on the basis of XDH phenotypic frequencies, at all seasons (Table 8).

(2) Seasonal Variation In Malate Dehydrogenase.

(Figures 13 and 14; Tables 6, 7, and 9)

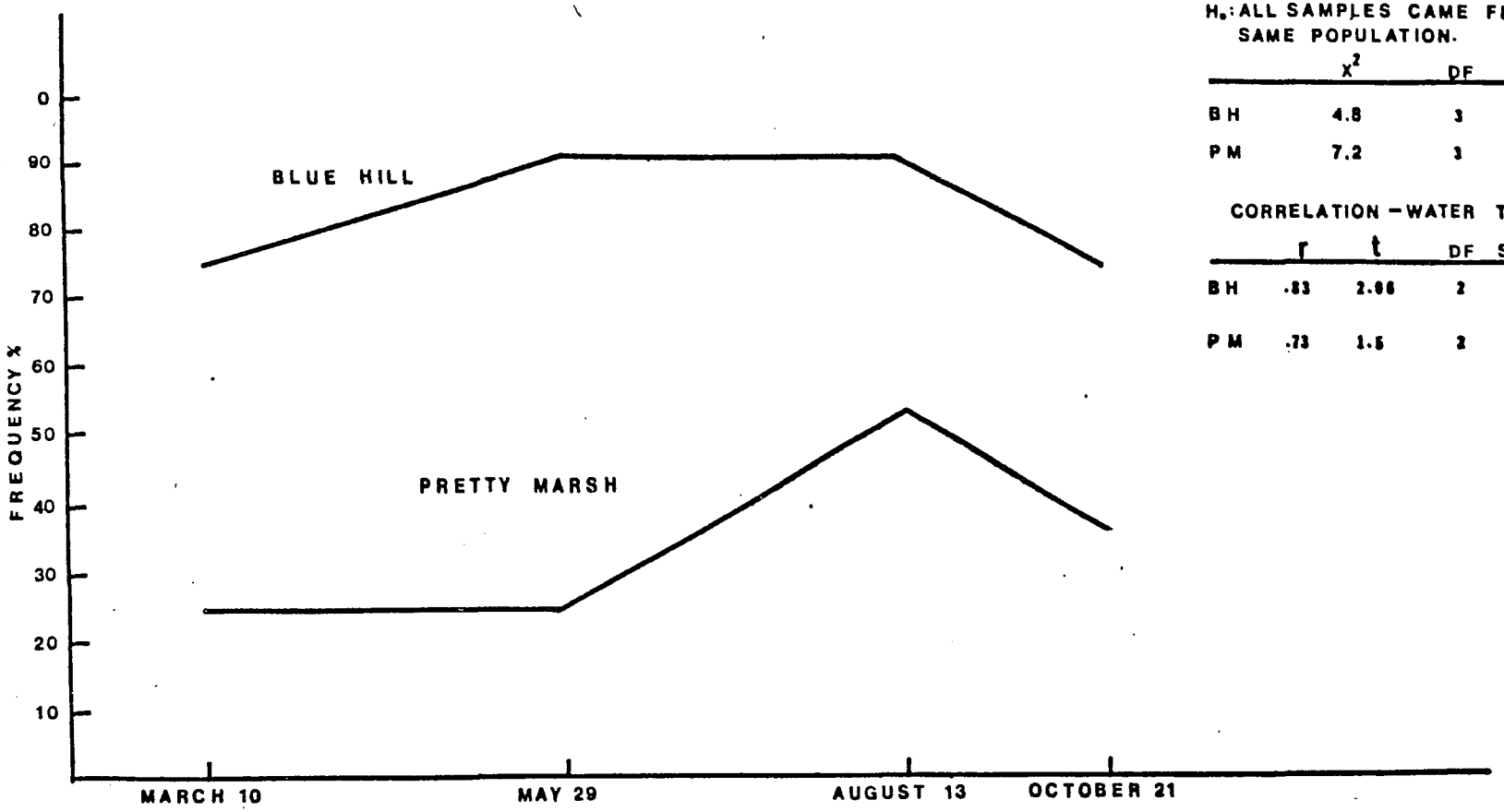
Electrophoretic examination of caecal tissues of specimens collected

FIGURE 12

Seasonal variation in the frequencies of the .36-.40 xanthine dehydrogenase phenotype at Blue Hill Falls and at Pretty Marsh.

SEASONAL ISOZYME VARIATION

XDH .36–.40 PHENOTYPE



HETEROGENEITY CHI SQUARE
H_o: ALL SAMPLES CAME FROM THE SAME POPULATION.

	X^2	DF	P
BH	4.8	3	<.25
PM	7.2	3	<.10

CORRELATION – WATER TEMR

	r	t	DF	SIGNIFICANT
BH	.83	2.86	2	NO
PM	.73	1.5	2	NO

TABLE 6

Seasonal Isozyme Variation At Blue Hill Falls:
A Summary Of Chi-Square Analyses

Enzyme System	X^2	DF	P
Xanthine Dehydrogenase	4.8	3	< .25
Malate Dehydrogenase	12.5	3	< .01
Alkaline Phosphatase	54.8	3	<.001
Acid Phosphatase	19.06	3	<.001

TABLE 7

Seasonal Isozyme Variation At Pretty Marsh:
A Summary Of Chi-Square Analyses

Enzyme System	X^2	DF	P
Xanthine Dehydrogenase	7.2	3	< .1
Malate Dehydrogenase	8.04	3	< .05
Alkaline Phosphatase	38.78	3	< .001
Acid Phosphatase	22.82	3	< .001

TABLE 8

Seasonal Variation In XDH Phenotypic Frequencies At Blue Hill Falls And Pretty Marsh: A Summary Of Chi-Square Analyses

Season	X^2	DF	P
March	12	1	< .001
May	22	1	< .001
August	7.86	1	< .01
October	8.4	1	< .005

from the salt pond at Blue Hill Falls during March, May, August, and October of 1980 reveals the same seven MDH phenotypes as were detected in the study of geographic variation (Section A3 of Results, Figure 9). Not all phenotypes were demonstrable during all four seasons of sampling. As was noted in Section A3 of Results, the 2 and 3A phenotypes were present in the highest frequencies, and the 3B, 3C, 3D, 4A and 4B, if present at all, were in low frequency. For the same statistical reasons noted in Section A3 of Results, these phenotypes were not analyzed. The frequency of the 3A phenotype rises from a low in March to a maximum in August, then decreases in October. The frequencies of the 2 phenotype do not seem to display a consistent pattern of seasonally related change (Figure 13). A contingency chi-square test shows that the four sampling seasons differ significantly on the basis of the 2 and 3A MDH phenotypic frequencies (Table 6). While the pattern of seasonal change in the frequencies of the 3A phenotype parallels that of seasonal change in sea water temperatures, the correlation is not significant. Similarly, at Pretty Marsh the frequency of the 3A phenotype rises from a low in March to a maximum in August, then decreases in October (Figure 14). A significant correlation ($r = .98$) of the 3A frequencies with seasonal change in the temperatures of seawater exists at Pretty Marsh. As at Blue Hill Falls, the frequencies of the 2 phenotype do not show a consistent pattern of seasonal variations. A contingency chi-square test shows that the sampling seasons differ significantly on the basis of the two MDH phenotypes (Table 7). A contingency chi-square test shows that the two sampling locations do not differ significantly on the basis of the 2 and 3A MDH phenotypic frequencies (Table 9).

FIGURE 13

Seasonal variation in the relative frequencies of individuals displaying 2 and 3A MDH phenotypes at Blue Hill Falls.

SEASONAL ISOZYME VARIATION

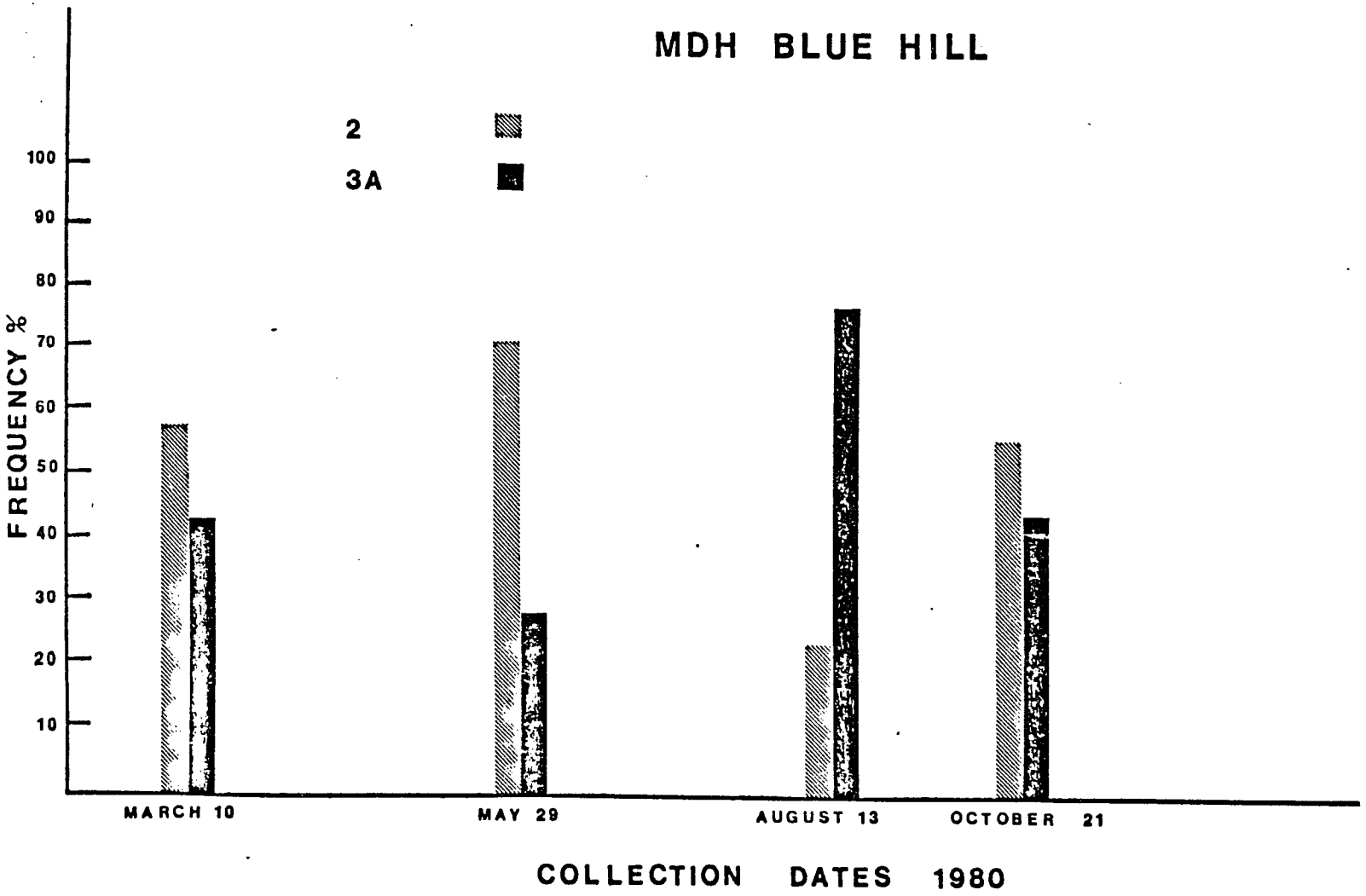


FIGURE 14

Seasonal variation in the relative frequencies of individuals displaying 2 and 3A MDH frequencies at Pretty Marsh.

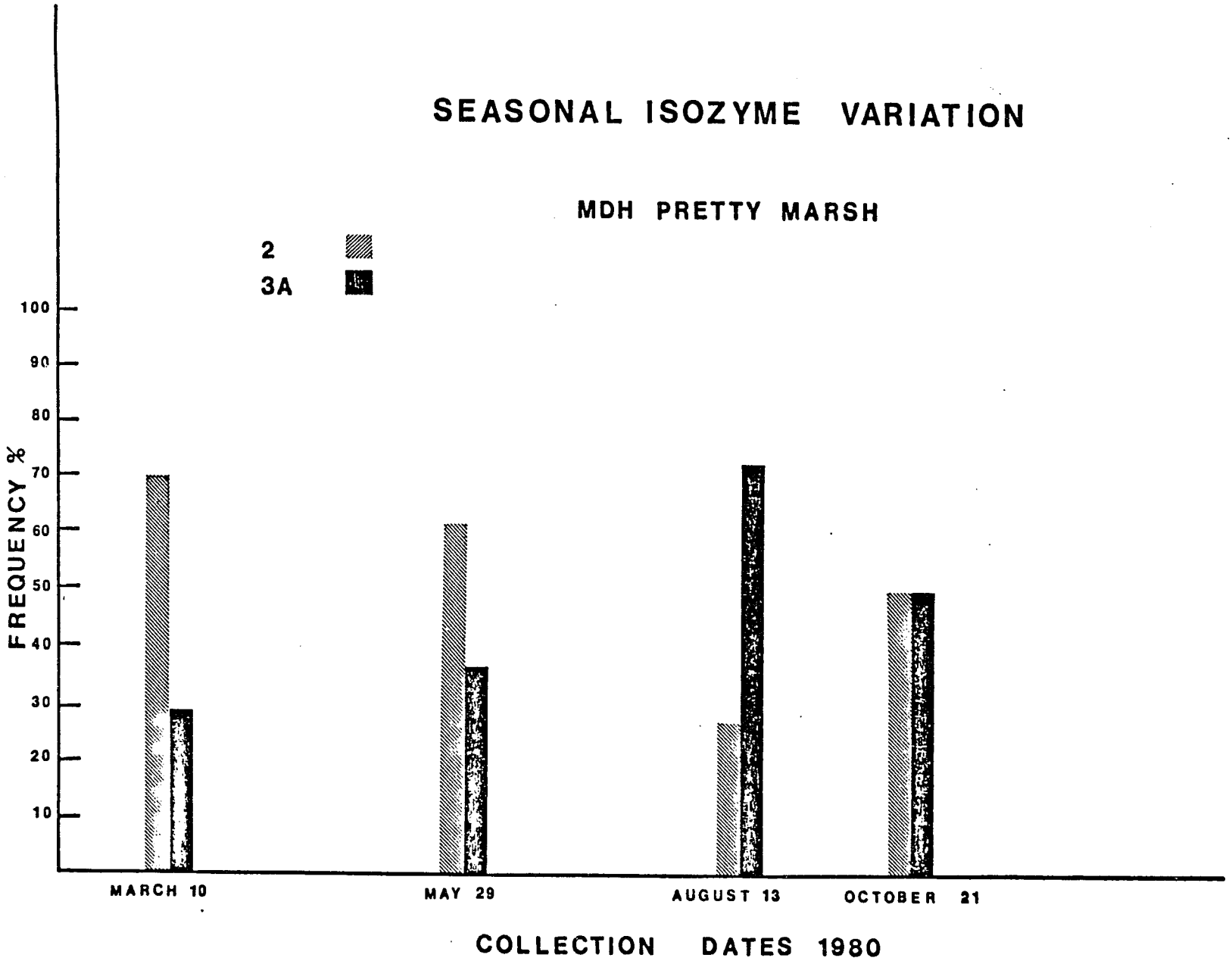

SEASONAL ISOZYME VARIATION
MDH PRETTY MARSH
2
3A
100
90
80
70
60
50
40
30
20
10
FREQUENCY %
MARCH 10
MAY 29
AUGUST 13
OCTOBER 21
COLLECTION DATES 1980

TABLE 9

Seasonal Variation In MDH Phenotypic Frequencies At Blue Hill Falls And Pretty Marsh: A Summary Of Chi-Square Analyses

Season	X^2	DF	P
March	.5	1	< .5
May	.39	1	< .75
August	.12	1	< .75
October	.19	1	< .75

(3) Seasonal Variation In Phosphatases.

(Figures 15 through 27; Tables 10, 11, and 12)

General observations on seasonal variation in the occurrence of acid and alkaline phosphatases at Blue Hill Falls and at Pretty Marsh suggest that results for these two enzyme systems be analyzed in two different ways. These observations are:

> All individuals sampled did not always display detectable levels of phosphatase activity. For example, during certain seasons, in any given sample there were those individuals which displayed phosphatase activity, and those for which no activity could be detected by the techniques employed. Moreover, those individuals which showed phosphatase activity displayed a range of band staining intensities from very light to dark. These conditions may be attributable to quantitative differences in the amount of a particular phosphatase isozyme present in the caecal tissues at that time.
>
> Both acid and alkaline phosphatases displayed a varied array of banding patterns with differing mobilities. These bands and combinations of bands could not be readily assigned to consistent phenotypic classes.

Two different approaches are employed in the presentation of results from both acid and alkaline phosphatases. In one approach, results are presented in terms of the numbers of

individuals displaying enzyme activity, regardless of individual band mobility and number of bands present in any given individual. In a second approach, an attempt is made to present results as has been done in forgoing sections; that is, to identify phenotypic classes based on band mobilities and combinations of bands, and then to assign individuals to those phenotypic classes. These two approaches are each applied separately to alkaline phosphatase and to acid phosphatase.

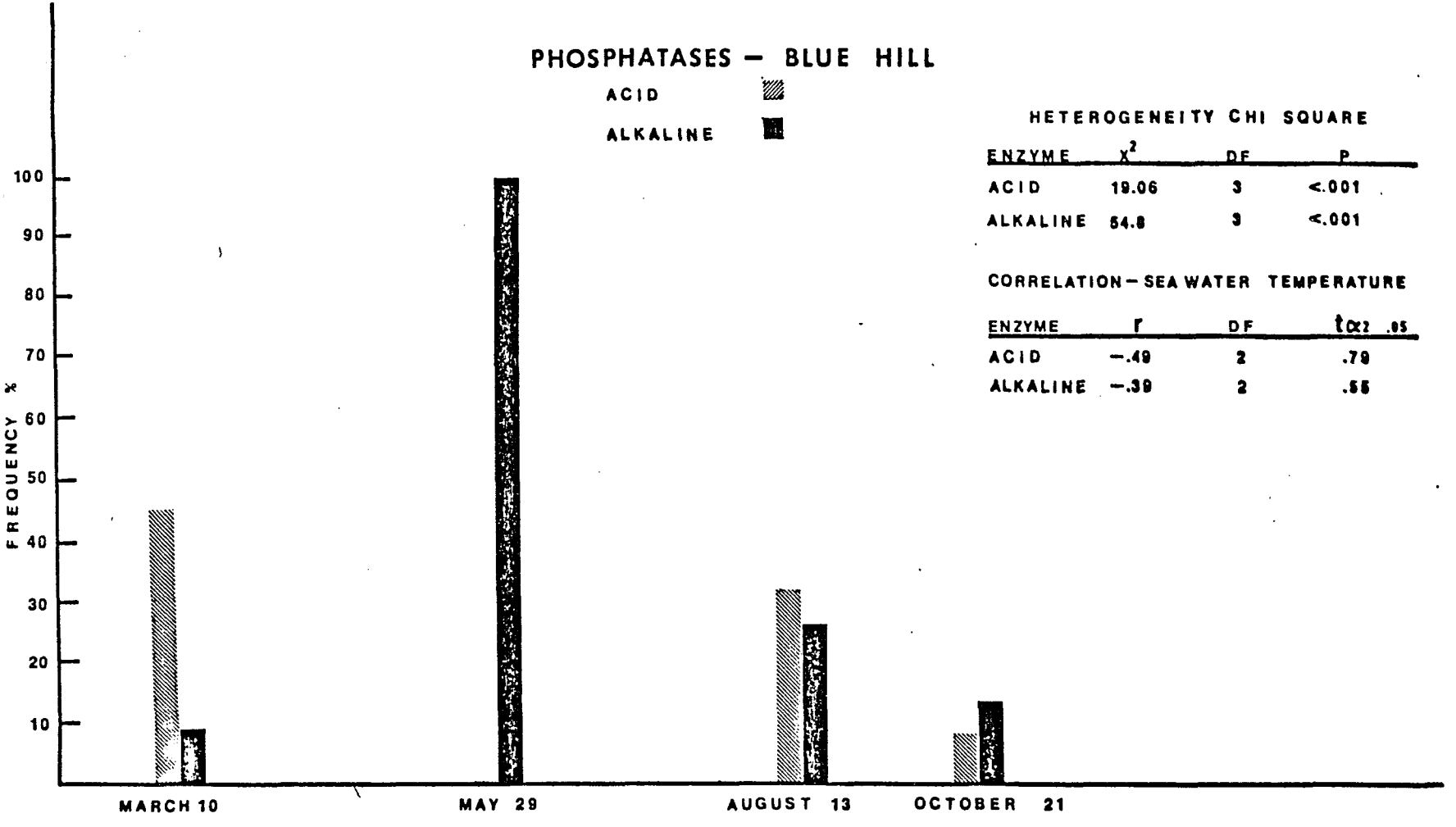
(a) Seasonal Variation In The Presence Of Alkaline Phosphatase. (Figure 15)

Electrophoretic examination of caecal tissues of specimens collected from the salt pond at Blue Hill Falls during March, May, August, and October, 1980, shows the following pattern of seasonally related variation. The number of individuals that have electrophoretically detectable, alkaline phosphatase activity is low in March (8%-2/24), rises to a maximum of 100% (24/24) in May, declines to 25% (6/24) in August, and finally declines still further to 13% (3/24) in October (Figure 15, Table 6). Contingency chi-square analysis shows that the sampling seasons differ significantly on the basis of the presence of alkaline phosphatase activity (Table 6). At Pretty Marsh, unlike Blue Hill Falls, the number of individuals showing activity is high in March, 92% (22/24), remains high in May, 100% (24/24), drops in August to 29% (7/24), and rises slightly in October to 46% (11/24) (Figure 16). A contingency chi-square test demonstrates that the sampling seasons at Pretty Marsh differ significantly on the basis of the presence of alkaline phosphatase activity (Table 7). At both Blue Hill Falls and Pretty Marsh the data suggests the existence of seasonal

FIGURE 15

Seasonal variation in the relative frequencies of individuals displaying acid and alkaline phosphatase activity at the salt pond at Blue Hill Falls.

SEASONAL ISOZYME VARIATION



HETEROGENEITY CHI SQUARE

ENZYME	X^2	DF	P
ACID	19.06	3	<.001
ALKALINE	54.8	3	<.001

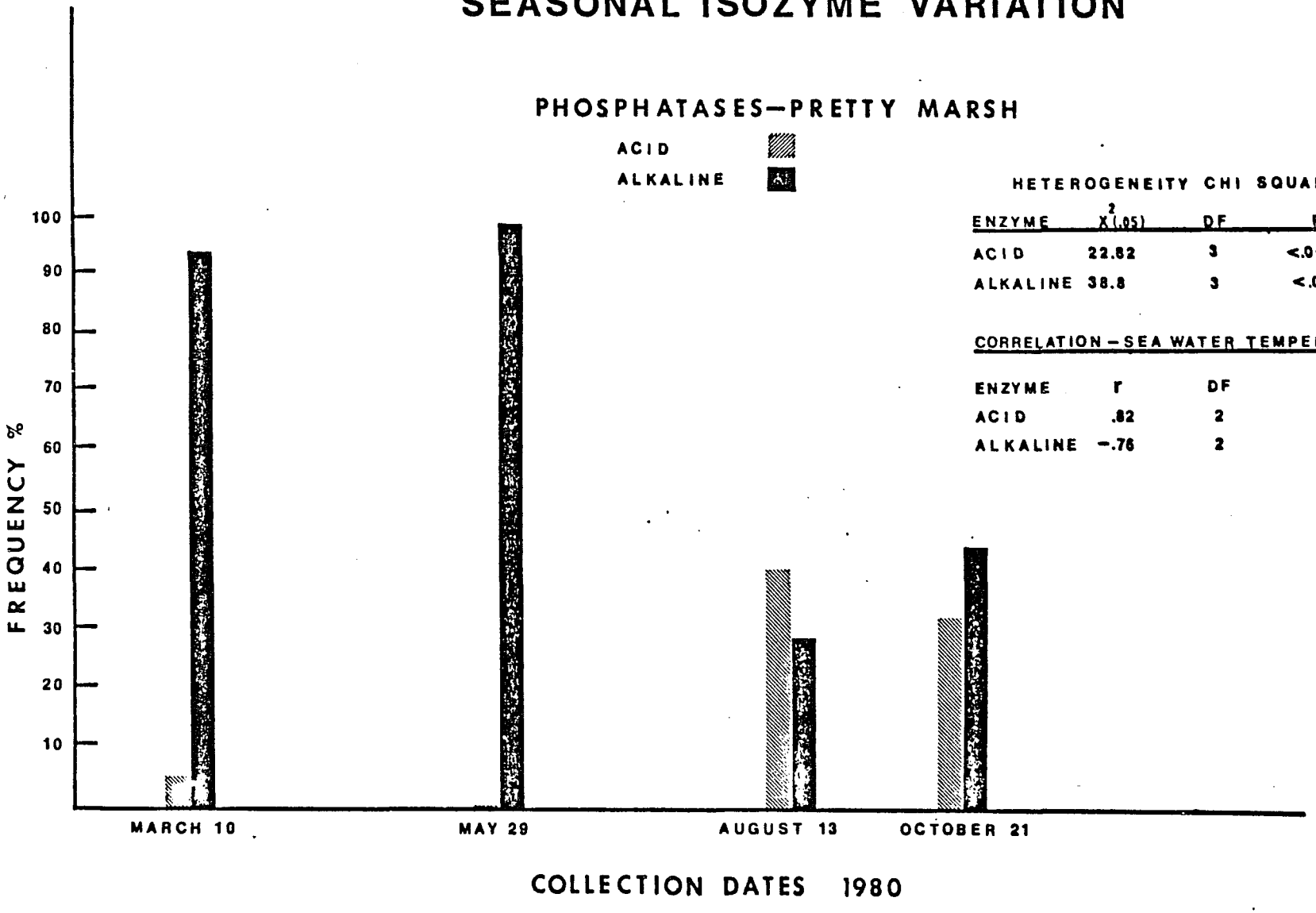
CORRELATION – SEA WATER TEMPERATURE

ENZYME	r	DF	$t_{\alpha/2\ .05}$
ACID	–.49	2	.79
ALKALINE	–.39	2	.55

FIGURE 16

Seasonal variation in the relative frequencies of individuals displaying acid and alkaline phosphatase activity at Pretty Marsh.

SEASONAL ISOZYME VARIATION



HETEROGENEITY CHI SQUARE

ENZYME	$\chi^2_{(.05)}$	DF	P
ACID	22.82	3	<.001
ALKALINE	38.8	3	<.001

CORRELATION – SEA WATER TEMPERATURE

ENZYME	r	DF	$t_{\alpha 2,.05}$
ACID	.82	2	2.02
ALKALINE	−.76	2	1.66

variation in terms of alkaline phosphatase activity. However, the cycle of variation is different at the two locations. During March, few animals at Blue Hill Falls show alkaline phosphatase activity, while at Pretty Marsh in the same month, a large number of animals display alkaline phosphatase activity. The difference between the locations during this month is significant according to a contingency chi-square test (Table 10). During both May and August no significant differences exist between the two populations. In October, however, significant differences exist between the two locations with regard to the presence of alkaline phosphase activity (Table 10). At either location no significant correlation exists between the frequency of individuals showing activity and seasonal change in the temperatures of seawater.

(b) Seasonal Variation In The Presence Of Acid Phosphatase.

(Figures 15 and 16)

Electrophoretic examination of caecal tissues of specimens collected from the salt pond at Blue Hill Falls during the seasons previously noted suggests the existence of a pattern of seasonal change in the presence of acid phosphatase (Figure 15). The number of individuals displaying activity is high in March, 50% (12/24), decreases in May to 0%, rises in August to 33% (8/24), and drops in October to 8% (2/24). The difference among seasons is statistically significant (Table 11). At Pretty Marsh, the number of individuals showing electrophoretically detectable acid phosphatase activity is low in March, 4% (1/24), remains low in May, 0% (0/24), but rises in August to 41% (10/24), and decreases in October to 33% (8/24) (Figure 16). As at Blue Hill Falls, the differences

TABLE 10

Seasonal Variation In The Presence Of Alkaline Phosphatase At Blue Hill Falls And Pretty Marsh: A Summary Of Chi-Square Analyses

Season	X^2	DF	P
March	33.2	1	< .001
May	0	1	.999
August	.106	1	< .75
October	4.68	1	< .05

TABLE 11

Seasonal Variation In The Presence Of Acid Phosphatase At Blue Hill Falls And Pretty Marsh: A Summary Of Chi-Square Analyses

Season	X^2	DF	P
March	14.2	1	< .001
May	0	1	.999
August	.35	1	< .5
October	4.54	1	< .05

among seasons are statistically significant (Table 7). A season-by-season comparison of the presence of acid phosphatase activity at both Blue Hill Falls and Pretty Marsh demonstrates statistically significant differences between the two populations during March and October (Table 11). No significant correlation exists between seasonal frequencies of individuals showing acid phosphatase activity and seasonal change in the temperature of seawater, at either location.

(c) Seasonal Variation In Alkaline Phosphatase Phenotypes.

(Figures 17 to 23; Table 12)

Electrophoretic examination of caecal tissues of specimens from Blue Hill Falls and Pretty Marsh does not reveal the existence of readily identifiable phenotypic classes whose frequencies display consistent patterns of seasonally related variation either within or between populations (Figures 17 to 22). Despite this situation, some observations can be made which may prove to be of biological significance. One observation is that there does seem to be some seasonal variation at both locations, in the number of phenotypes present (Table 12). At both locations, the number of phenotypes is low in March, rises to a maximum in May, then decreases again to equivalent numbers in August and October. No significant statistical difference in the numbers of phenotypes is noted between the populations. A second observation is that in both populations, the frequency of one particular band is high, near 90% in May. A final observation is the appearance in May in a few (4-6) individuals of both populations, of additional bands of low mobility, with Rf values of about .18, .24, and .33.

FIGURE 17

Variation in alkaline phosphatase phenotypes observed in March and May at Blue Hill Falls. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ALKALINE PHOSPHATASE BLUE HILL

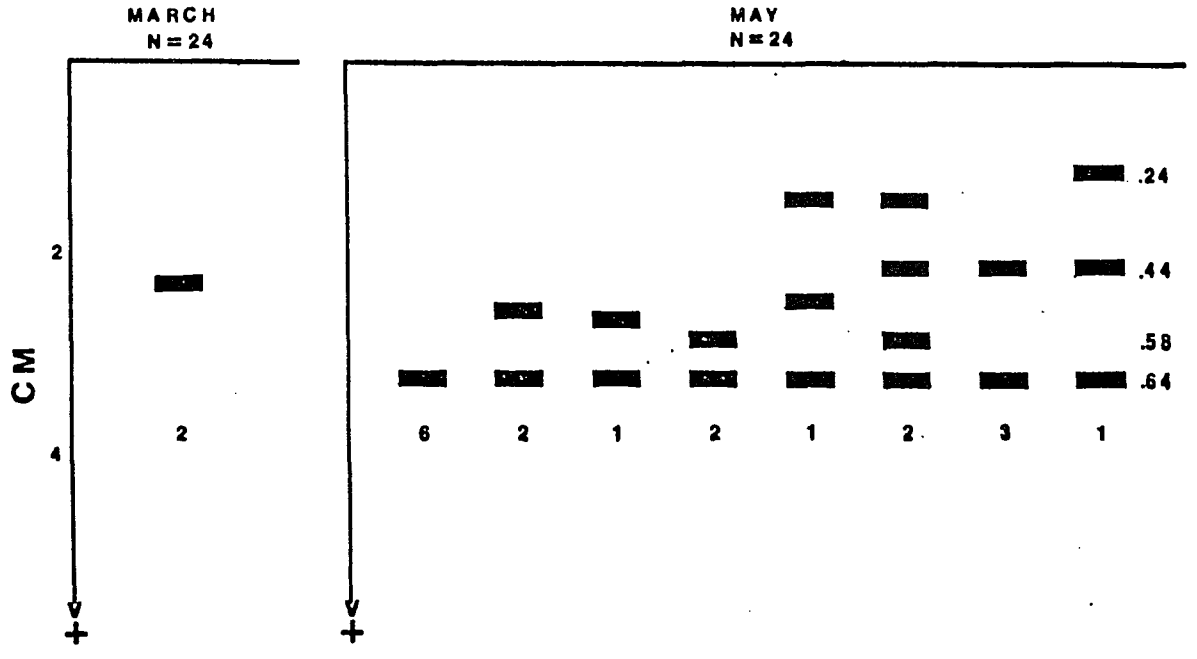


FIGURE 18

Variation in alkaline phosphatase phenotypes observed in August and October at Blue Hill Falls. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ALKALINE PHOSPHATASE BLUE HILL

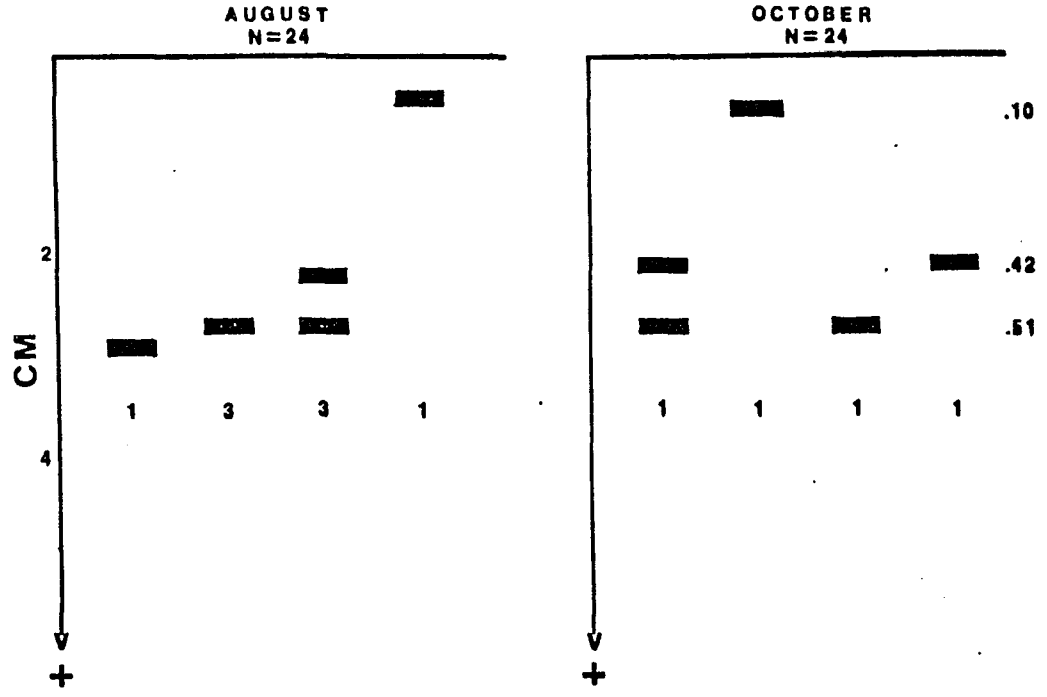


FIGURE 19

Variation in alkaline phosphatase phenotypes observed in March and May at Pretty Marsh. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ALKALINE PHOSPHATASE PRETTY MARSH

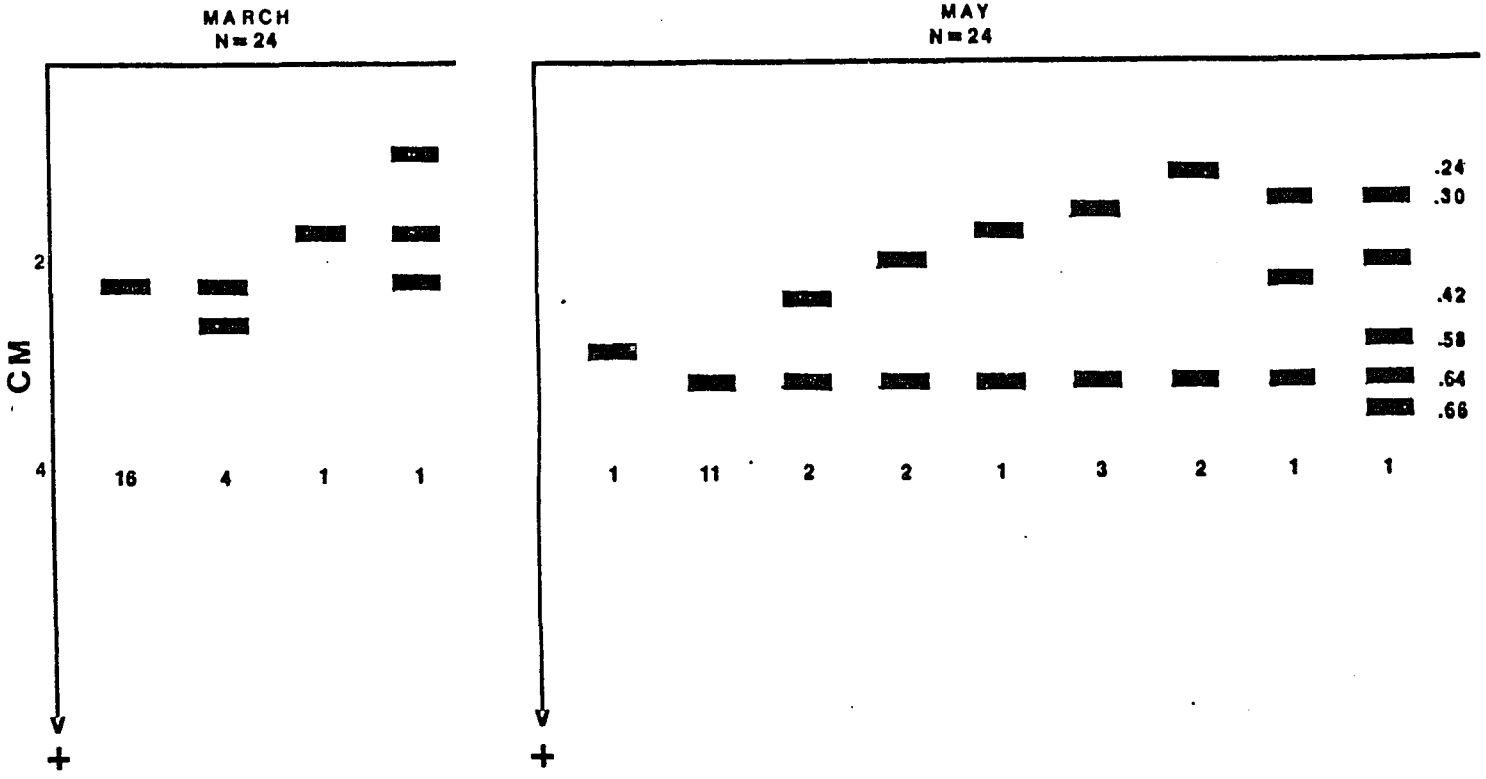


FIGURE 20

Variation in alkaline phosphatase phenotypes observed in August and October at Pretty Marsh. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ALKALINE PHOSPHATASE PRETTY MARSH

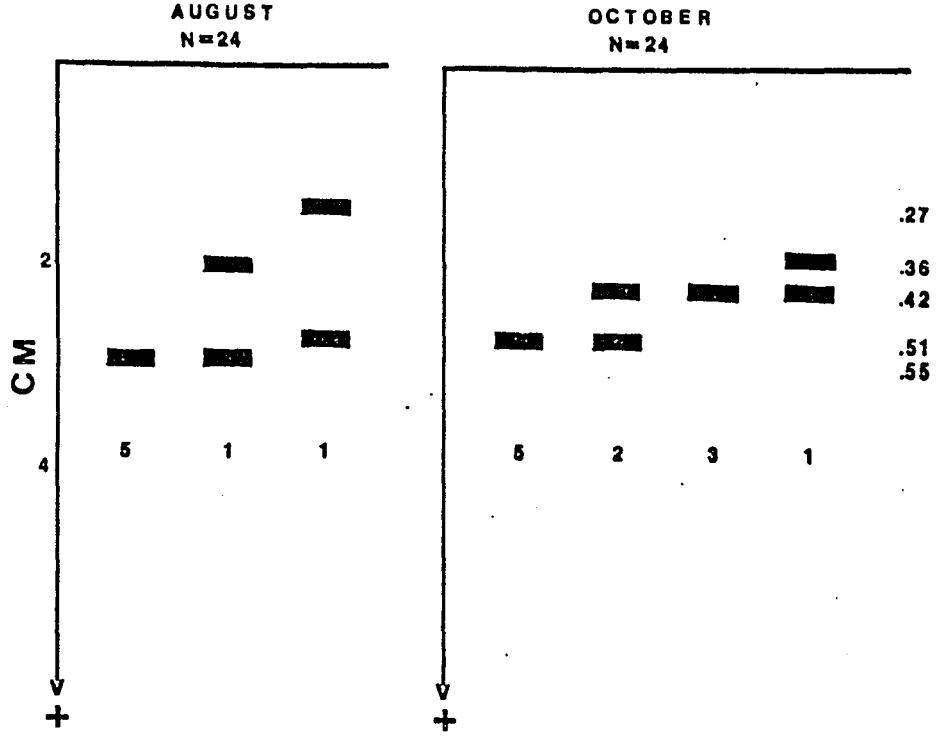


FIGURE 21

A photograph of a polyacrylamide gel showing alkaline phosphatase phenotypes during May at Blue Hill Falls and at Pretty Marsh.

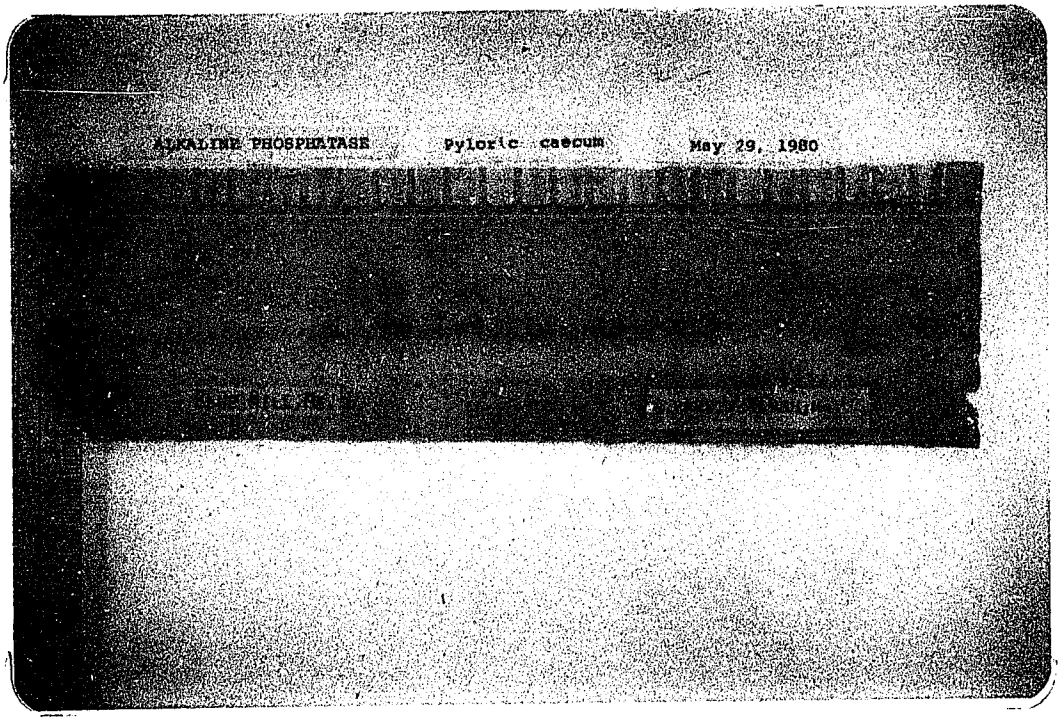

ALKALINE PHOSPHATASE
Pyloric caecum
May 29, 1980

FIGURE 22

A photograph of a polyacrylamide gel showing alkaline phosphatase phenotypes during August at Blue Hill Falls and at Pretty Marsh.

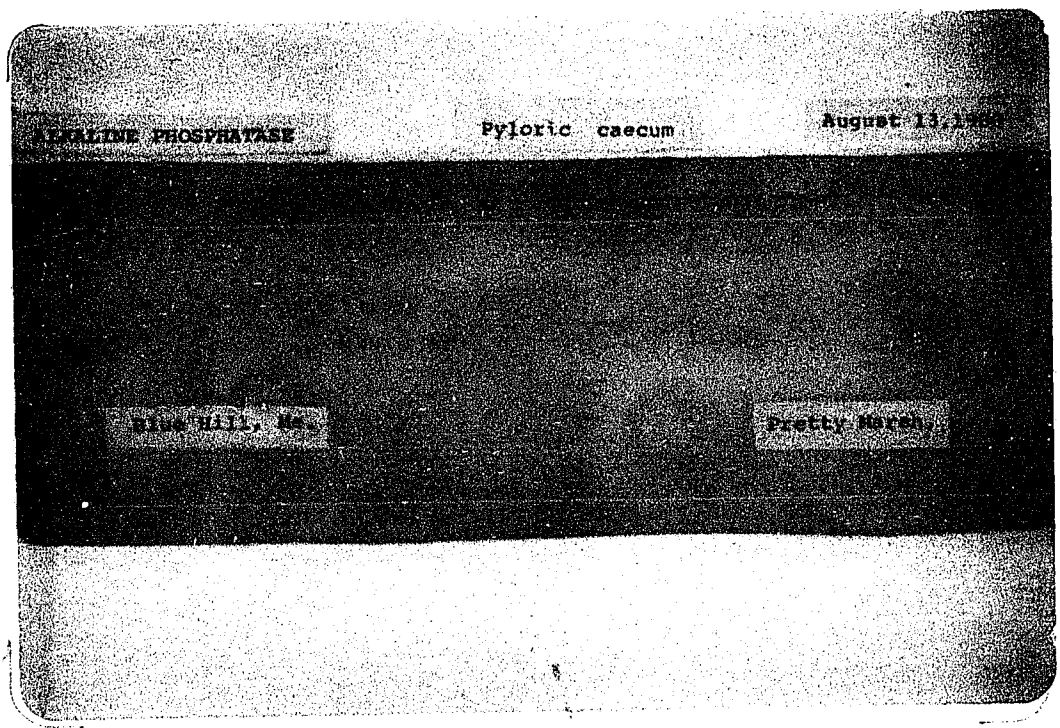

ALKALINE PHOSPHATASE
Pyloric caecum
August 13,
Pretty March,

TABLE 12

Seasonal Variation In the Number Of Alkaline Phosphatase Phenotypes Observed At Blue Hill Falls And Pretty Marsh

Season	# of Phenotypes Salt pond	Pretty Marsh
March	1	4
May	8	9
August	4	4
October	4	4

These bands appear to be less frequent during the months of March, August, and October.

(d) Seasonal Variation In Acid Phosphatase Phenotypes.

(Figures 23-27; Table 13)

Electrophoretic examination of caecal tissues of specimens from Blue Hill Falls and Pretty Marsh does not show the existence of readily identifiable acid phosphatase phenotypic classes, whose frequencies suggest consistent patterns of seasonal variation (Figures 23 to 27). As Table 13 shows, the number of acid phosphatase phenotypes at both locations is low in March, decreases to 0 in May, rises to a maximum in August, then decreases somewhat in October. No statistical analysis was performed because of the total absence of acid phosphatase in May.

(e) The Inverse Relationship Of The Frequencies Of Acid And Alkaline Phosphatase.

(Figures 15 and 16)

During the seasons examined, the frequency of individuals showing the presence of acid and alkaline phosphatase activity at any given location appears to be inversely related with respect to one another (Figures 15 and 16). When the number of individuals showing alkaline phosphatase activity is high, particularly in May, the number of individuals showing acid phosphatase activity is low. Over the seasons examined at Pretty Marsh, there is a significant negative correlation ($r = -.99$) between these two phosphatases. While such a negative correlation seems apparent by inspection at Blue Hill Falls, it is not statistically significant.

FIGURE 23

Variation in acid phosphatase phenotypes observed in March at Blue Hill Falls. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding patterns.

SEASONAL ISOZYME VARIATION

ACID PHOSPHATASE BLUE HILL

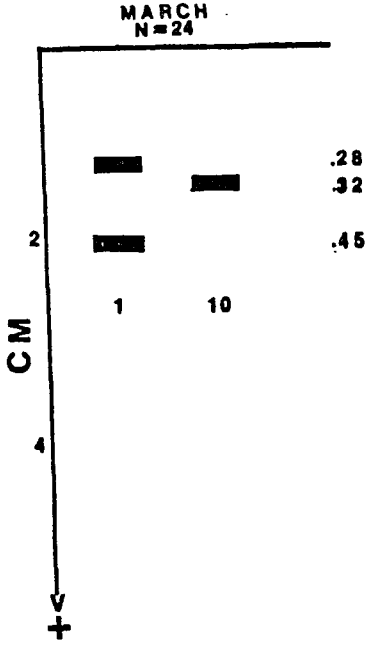


FIGURE 24

Variation in acid phosphatase phenotypes observed in August and October at Blue Hill Falls. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ACID PHOSPHATASE BLUE HILL

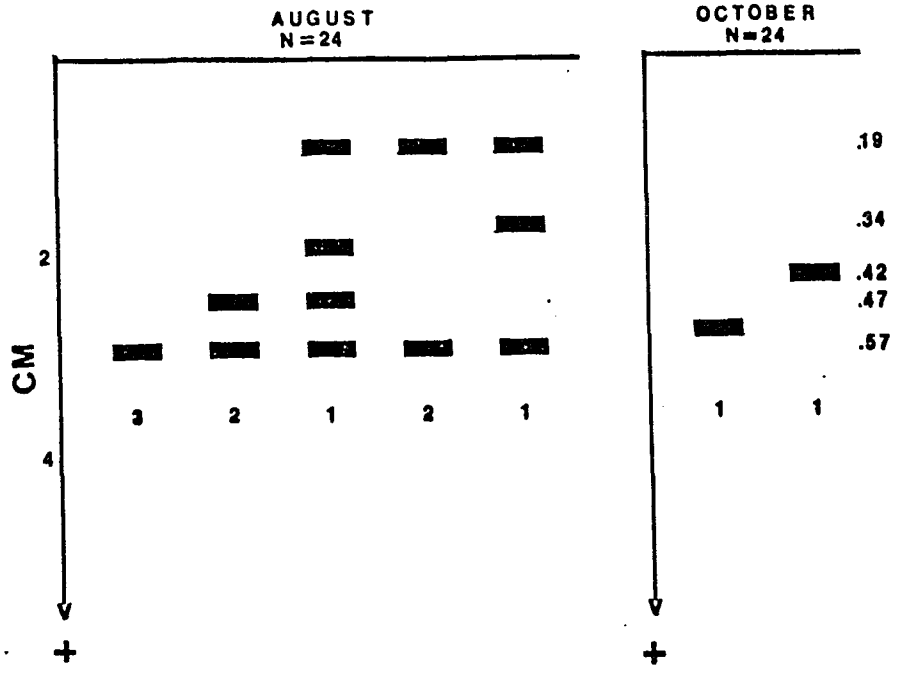


FIGURE 25

Variation in acid phosphatase phenotypes observed in March at Pretty Marsh. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ACID PHOSPHATASE PRETTY MARSH

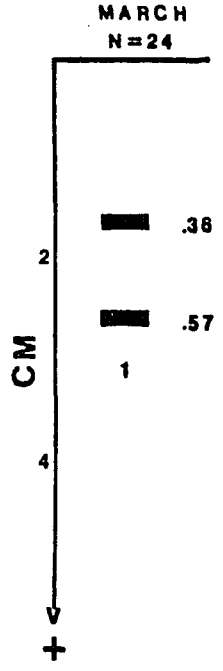


FIGURE 26

Variation in acid phosphatase phenotypes observed in August and October at Pretty Marsh. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ACID PHOSPHATASE PRETTY MARSH

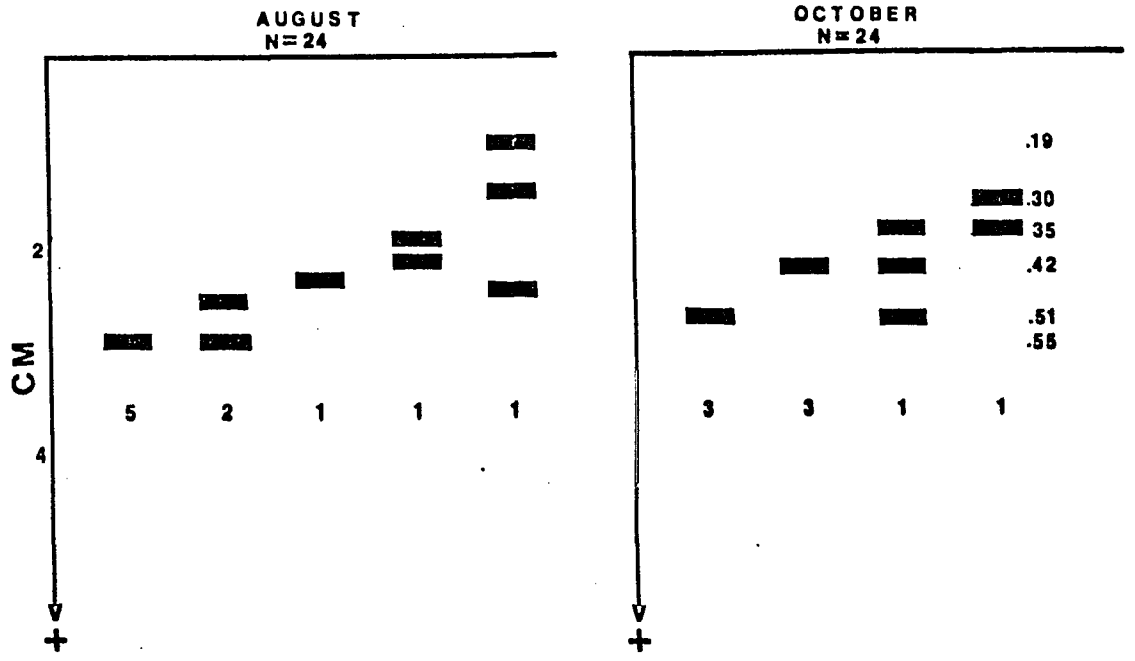


FIGURE 27

A photograph of a polyacrylamide gel showing acid phosphatase phenotypes during August at Blue Hill Falls and at Pretty Marsh.

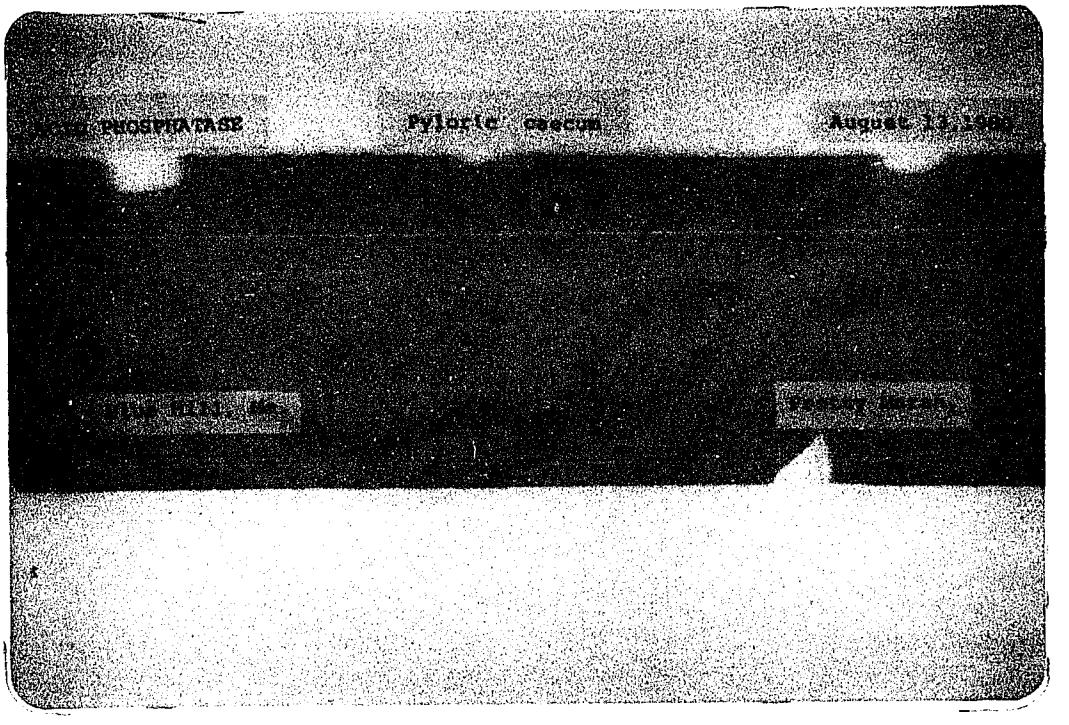

PHOSPHATASE
Pyloric caecum

TABLE 13

Seasonal Variation In The Number Of Acid Phosphatase Phenotypes Observed At Blue Hill Falls And At Pretty Marsh

Season	# of Phenotypes Salt Pond	Pretty Marsh
March	2	1
May	0	0
August	5	5
October	2	4

(4) Seasonal Variation In The Non-Specific Esterases.

(Figures 28 through 37; Table 13)

Electrophoretic analysis for non-specific esterases in specimens collected from Blue Hill Falls and Pretty Marsh during the seasons examined, reveals a large number of bands of differing mobilities. Various combinations of these bands result in a number of phenotypic classes. At both locations the number and variety of these phenotypes vary with season (Figures 28 through 37). Table 13 shows that phenotypes increase from a low (2-5) in March and May, to a maximum (19-23) in October. No statistically significant differences exist between the populations based on the number of phenotypes observed during the four seasons examined. At both Blue Hill Falls and Pretty Marsh, the frequencies of two specific combinations of bands appear to show patterns of seasonal variation which differ between the two locations. These two combinations of bands are a three banded complex (Group I) with Rf values of .45, .57, .64, and a two banded complex (Group II) with Rf values of .64 and .68. The bands of Group I are rather large and diffuse. Those with Rf values of .45 and .64 stain darkly; the band with an Rf of .57 stains very lightly (Figure 34). In Group II, the bands having Rf values of .64 and .68 are sharply defined, narrow, and stain darkly (Figure 35). Figure 36 shows that at Blue Hill Falls both Group I and Group II complexes are absent in March. In May the frequency of Group I increases to about 75%, while the frequency of Group II bands remains low. In August, observations show that the relative frequencies of the two groups reverse: the frequency of Group II is high, about 75%,

FIGURE 28

Variation in esterase phenotypes observed in March and May at Blue Hill Falls. Decimal numbers are relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ESTERASE PHENOTYPES–BLUE HILL

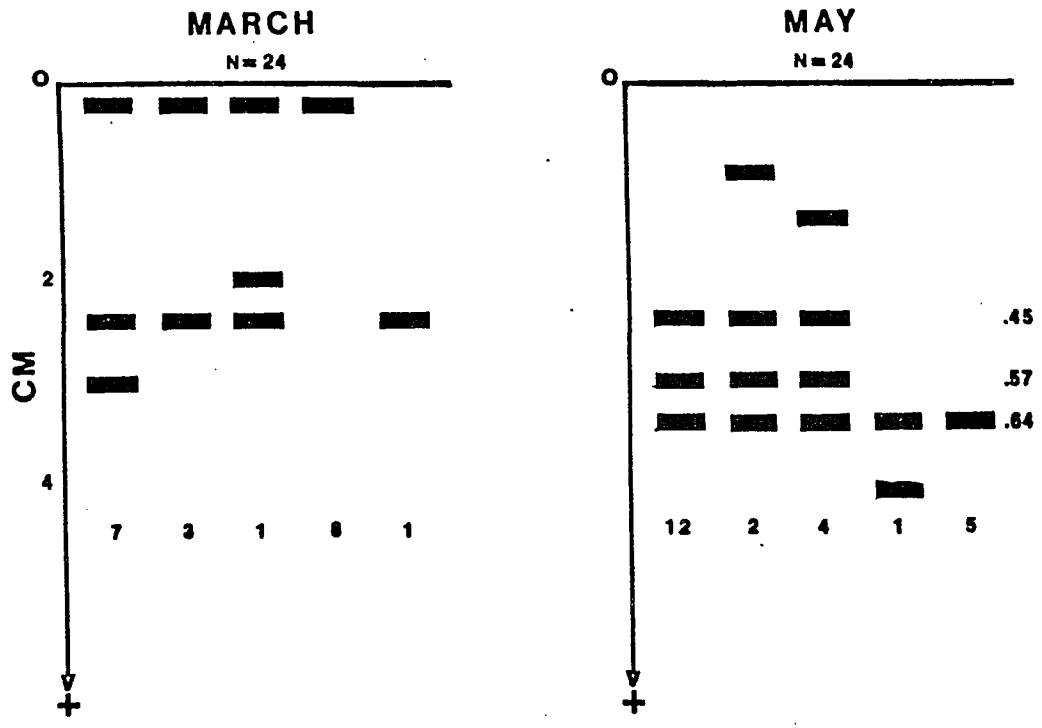


FIGURE 29

Variation in esterase phenotypes observed at Blue Hill Falls in August. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ESTERASE PHENOTYPES – BLUE HILL

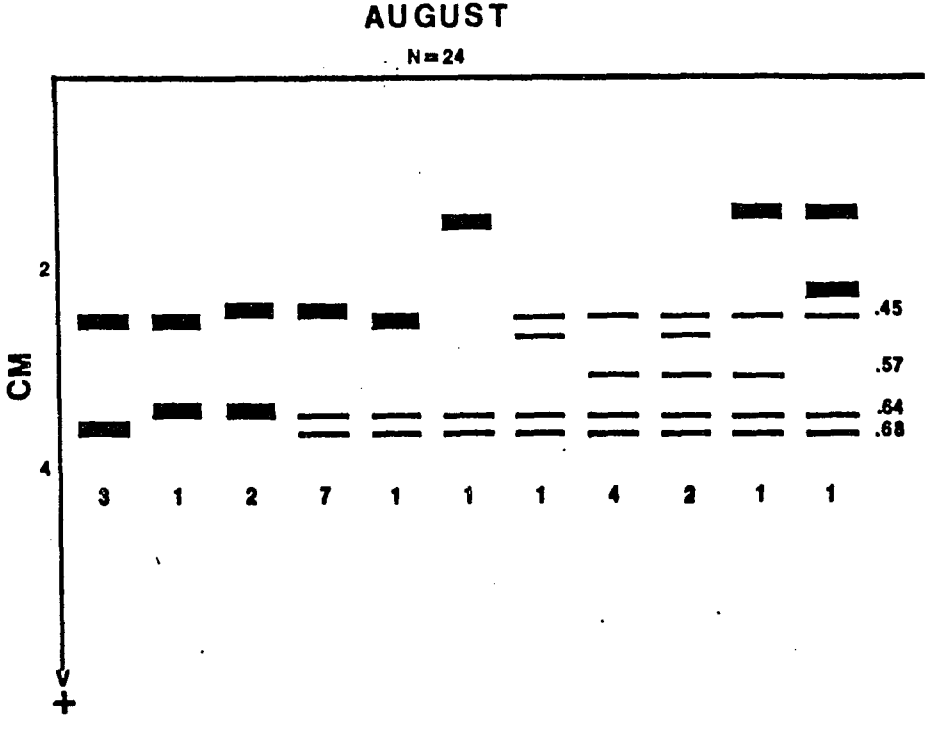


FIGURE 30

Variation in esterase phenotypes observed at Blue Hill Falls in October. Decimal numbers are relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below each banding pattern.

SEASONAL ISOZYME VARIATION

ESTERASE PHENOTYPES – BLUE HILL

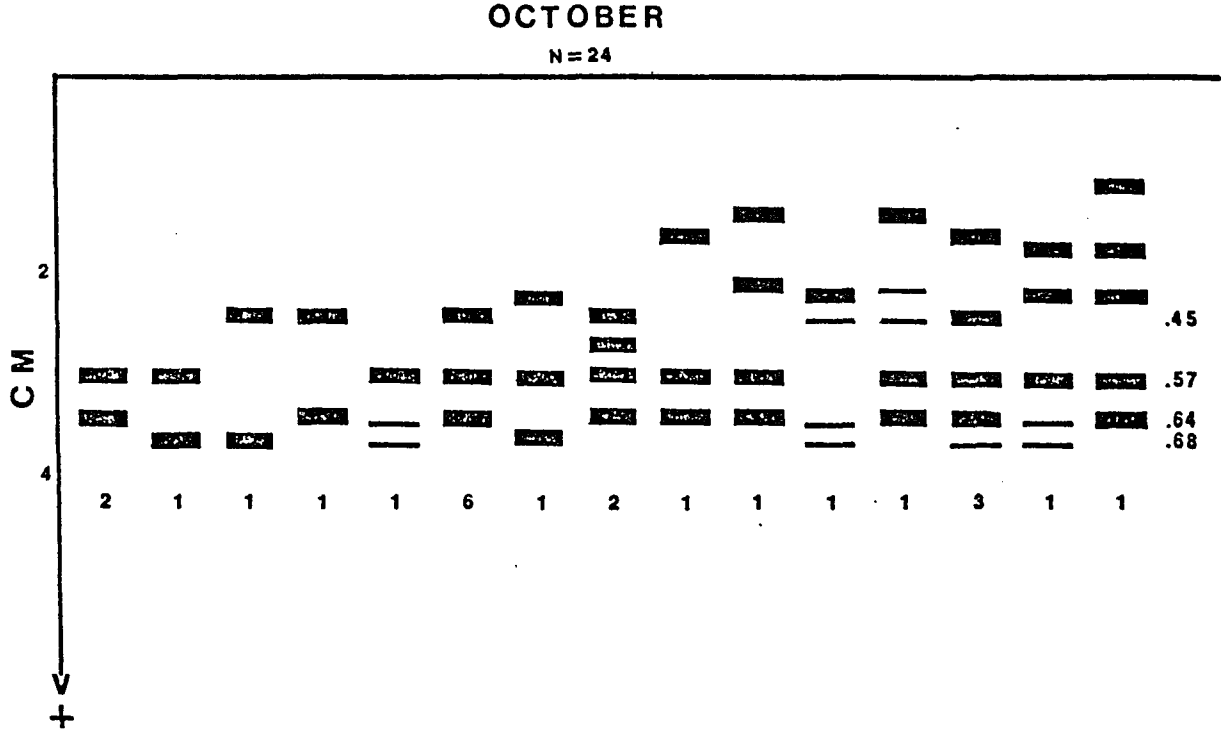


FIGURE 31

Variation in esterase phenotypes observed at Pretty Marsh in March and May. Decimal numbers are selective mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ESTERASE PHENOTYPES—PRETTY MARSH

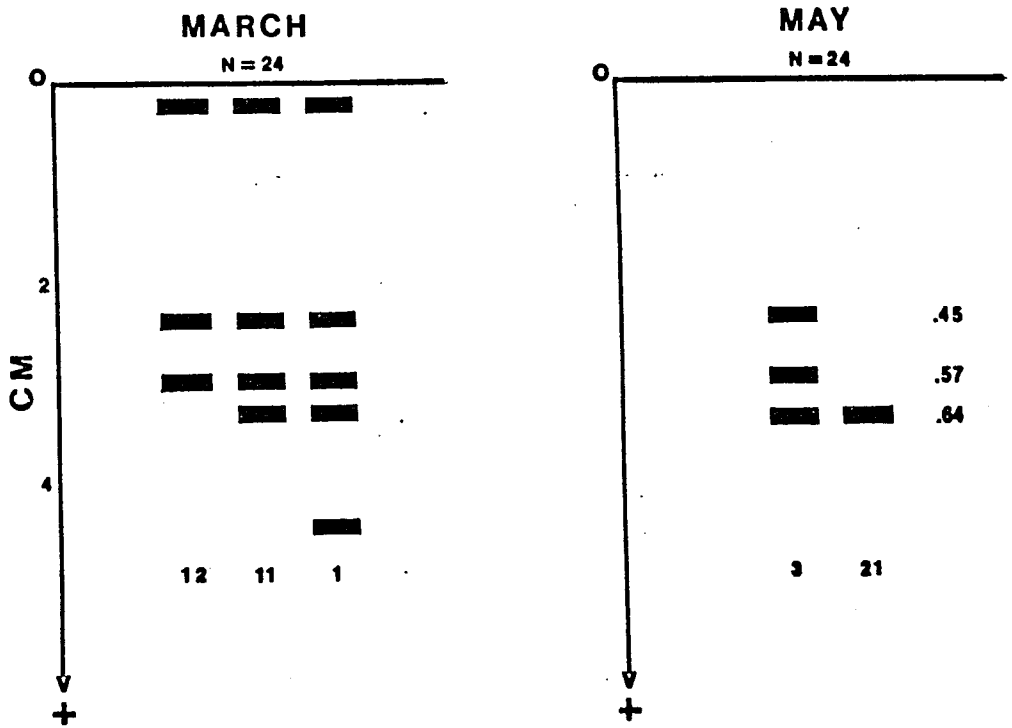


FIGURE 32

Variation in esterase phenotypes observed in August at Pretty Marsh. Decimal numbers are relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ESTERASE PHENOTYPES PRETTY MARSH

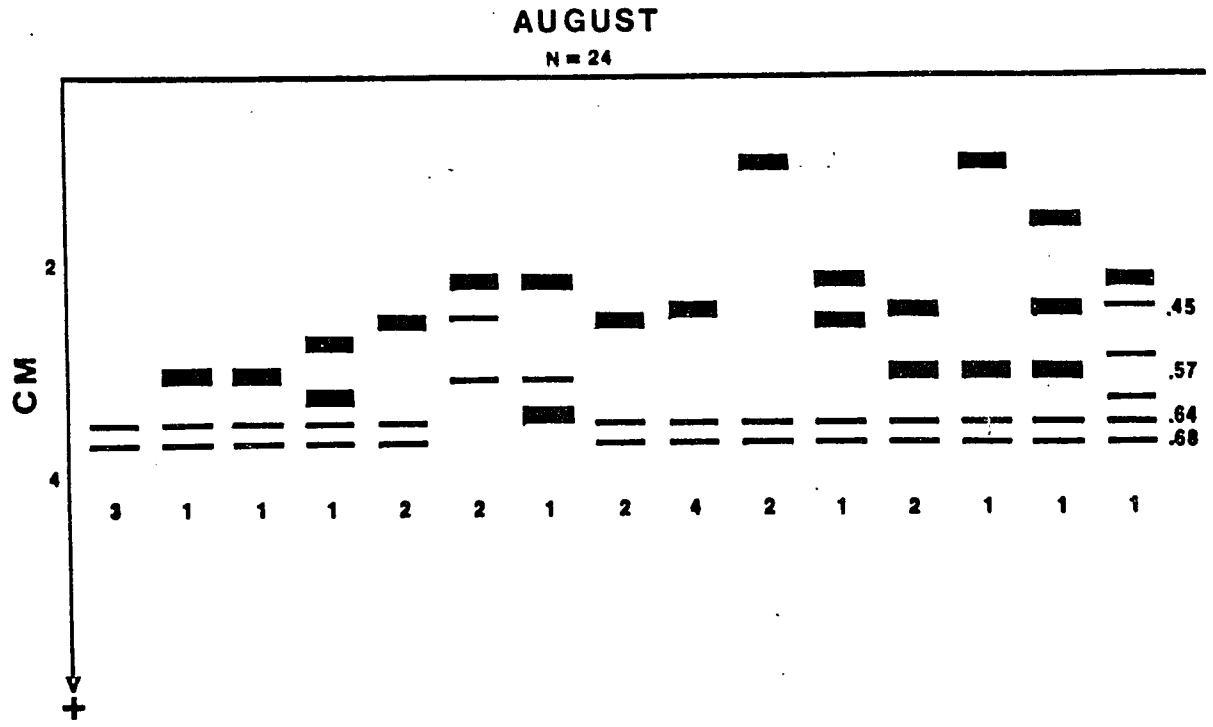


FIGURE 33

Variation in esterase phenotypes observed in October at Pretty Marsh. Decimal numbers are the relative mobilities of individual bands. Numbers of individuals of each phenotype are noted below the appropriate banding pattern.

SEASONAL ISOZYME VARIATION

ESTERASE PHENOTYPES-PRETTY MARSH

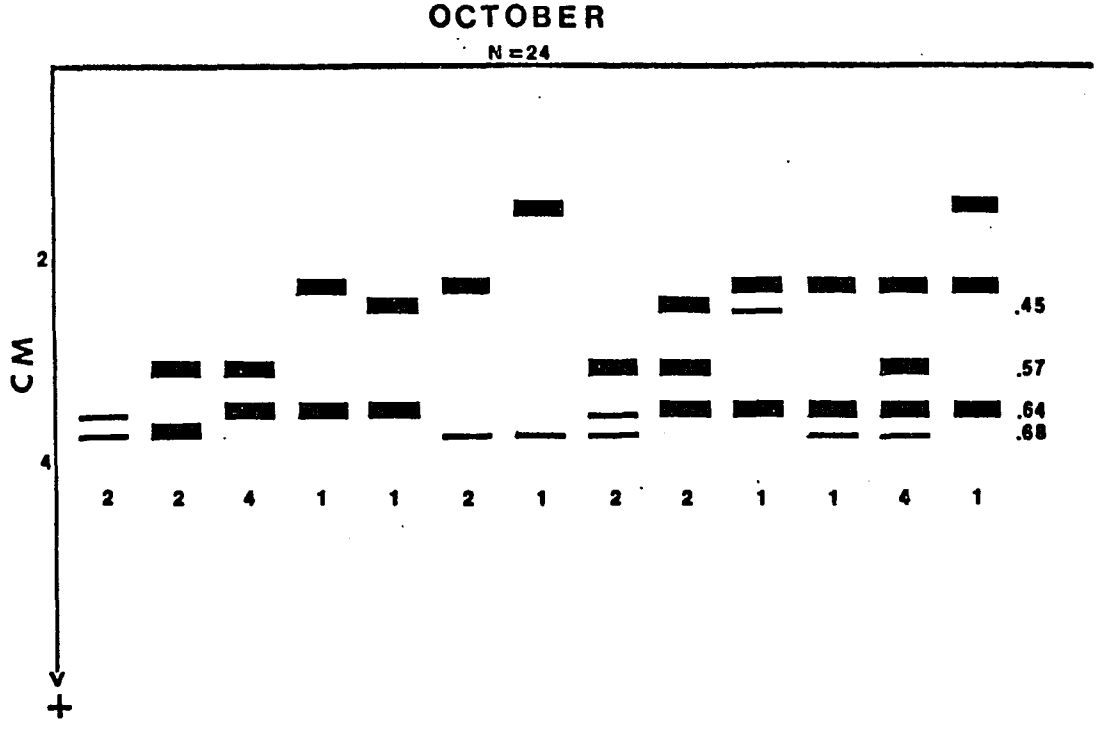


FIGURE 34

A photograph of a polyacrylamide gel showing esterase phenotypes observed in May in animals from Blue Hill Falls and from Pretty Marsh.


Pyloric caecum
May 29, 1980

FIGURE 35

A photograph of a polyacrylamide gel showing esterase phenotypes observed in August in animals from Blue Hill Falls and from Pretty Marsh.

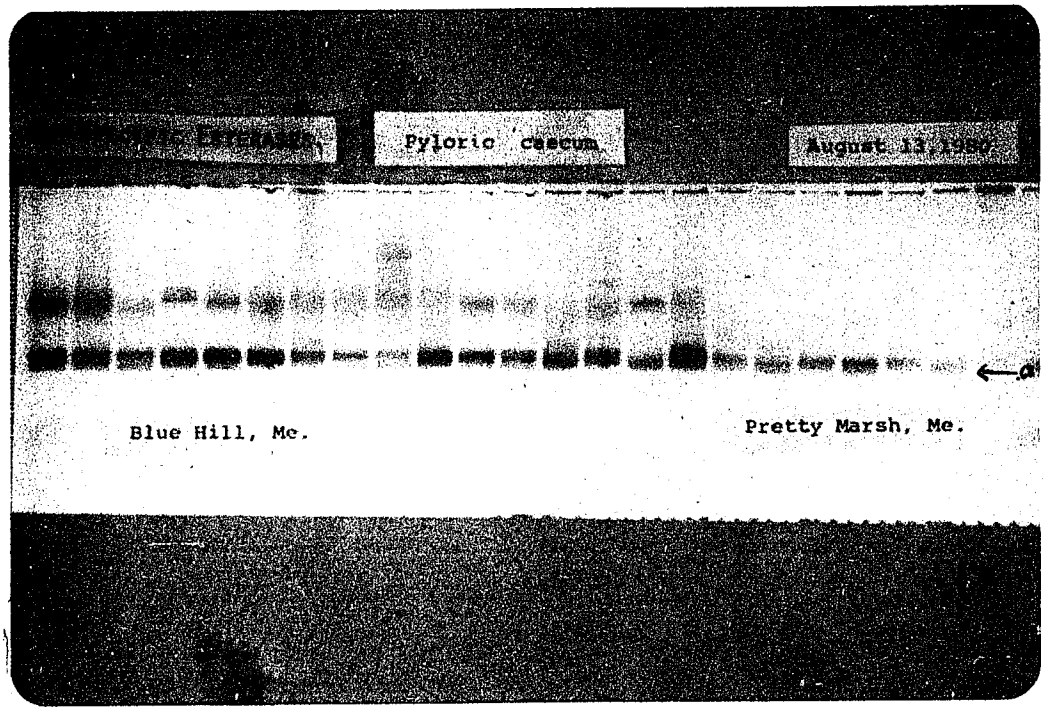

Pyloric caecum
Blue Hill, Me.
Pretty Marsh, Me.

TABLE 14

Seasonal Variation In The Number Of Different Esterase Phenotypes Observed At Blue Hill Falls And Pretty Marsh

Season	Location	
	Blue Hill Falls	Pretty Marsh
March	5	3
May	5	2
August	14	17
October	23	19

while the Group I isozymes are absent. In October the frequencies of both groups are equal at 25%. Specimens from Pretty Marsh (Figure 37) show the same general pattern of variation, but with some differences. The frequencies of the Group I isozymes are lower than at Blue Hill Falls, while the frequencies of Group II isozymes are higher than at Blue Hill Falls.

(5) Seasonal Variation In Leucine Aminopeptidase.

(Figure 38)

At both locations, leucine aminopeptidase is tentatively interpreted as being represented by four loci. One of those loci, the least mobile, appears to be polymorphic. Staining and grouping patterns suggest that this locus has two different co-dominant alleles, which assort in a Mendelian fashion to give three genotypic classes (Figure 38). At both locations, the S/F heterozygote is far more abundant than either homozygote. At Blue Hill Falls, chi-square analysis, noted in Table 14, shows significant deviations from Hardy-Weinberg expectations during all four sampling seasons. At Pretty Marsh, however, chi-square analysis (Table 15) indicates that the population is in Hardy-Weinberg equilibrium during March, May, and October. Only during August do genotypic frequencies display significant deviations from Hardy-Weinberg expectations.

C. The Effects Of Feeding And Fasting On Isozymes

This experiment was designed to test the effects of feeding and fasting on the enzyme systems used in the study of geographic variation and in the study of seasonal variation. Since none of

FIGURE 36

Seasonal variation in the frequencies of Group I and Group II esterase band complexes of animals from Blue Hill Falls.

SEASONAL ISOZYME VARIATION

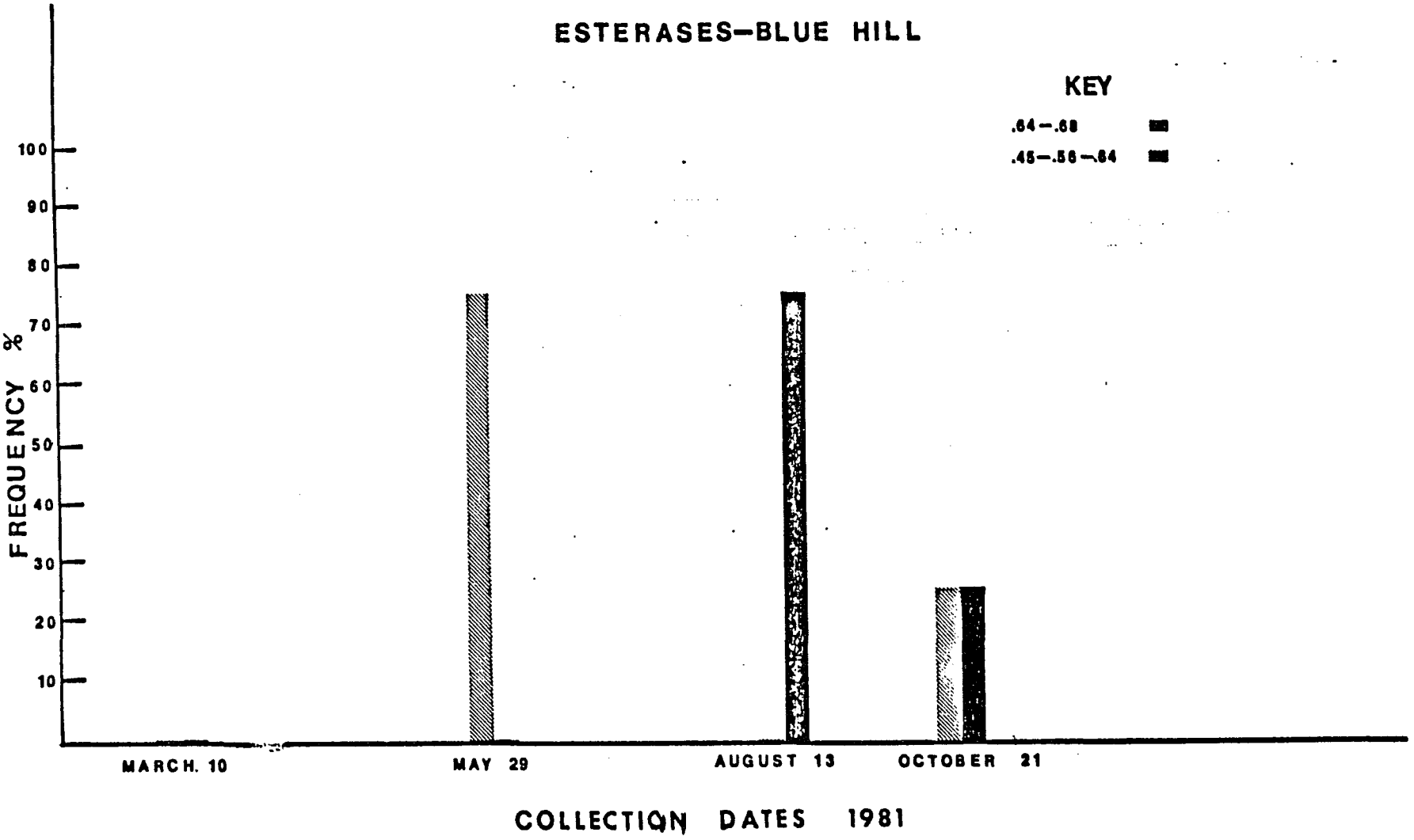


FIGURE 37

Seasonal variation in the frequencies of Group I and Group II esterase band complexes from animals collected at Pretty Marsh.

SEASONAL ISOZYME VARIATION

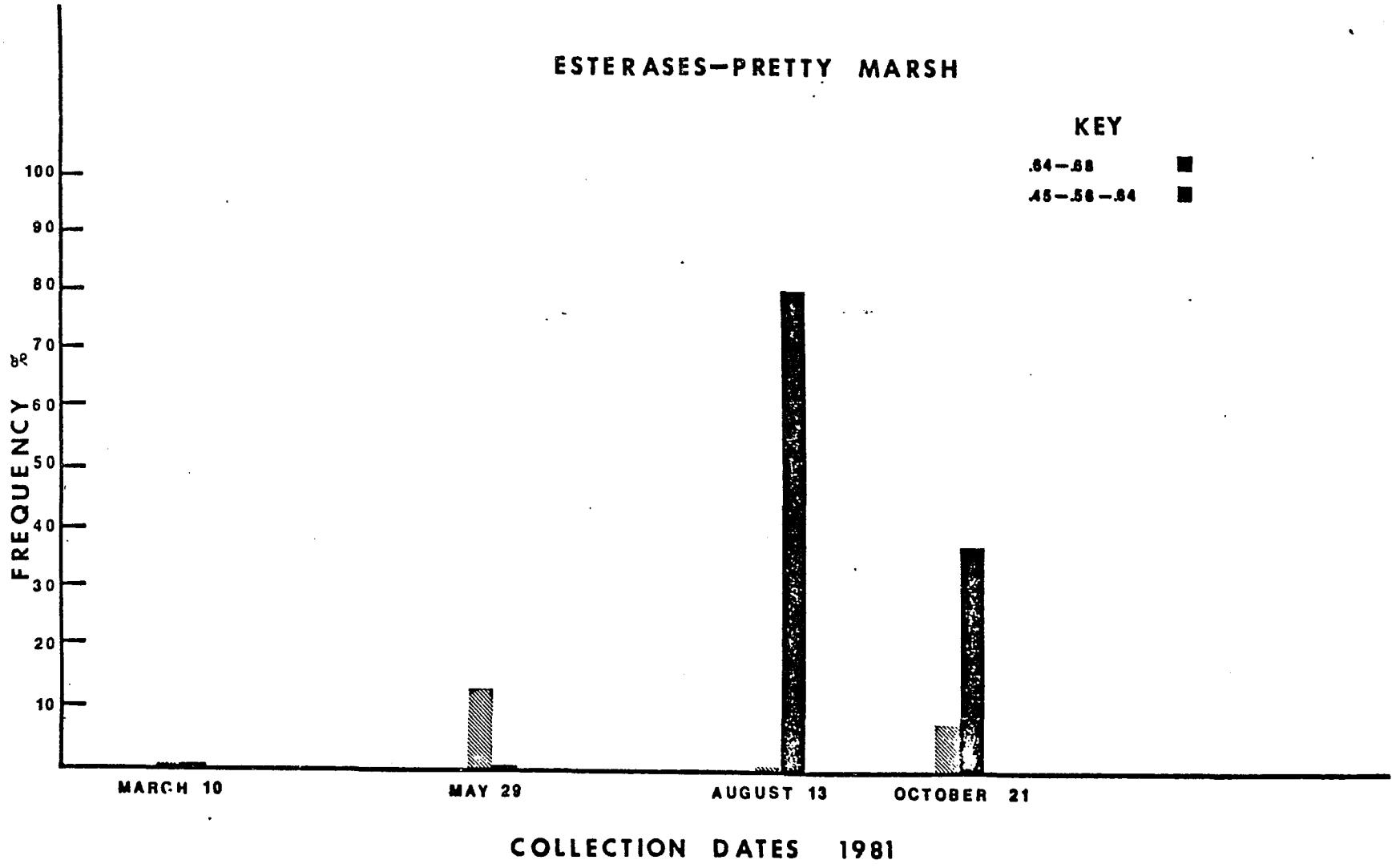


FIGURE 38

Three genotypic classes at a leucine aminopeptidase locus.

A SINGLE LOCUS MODEL

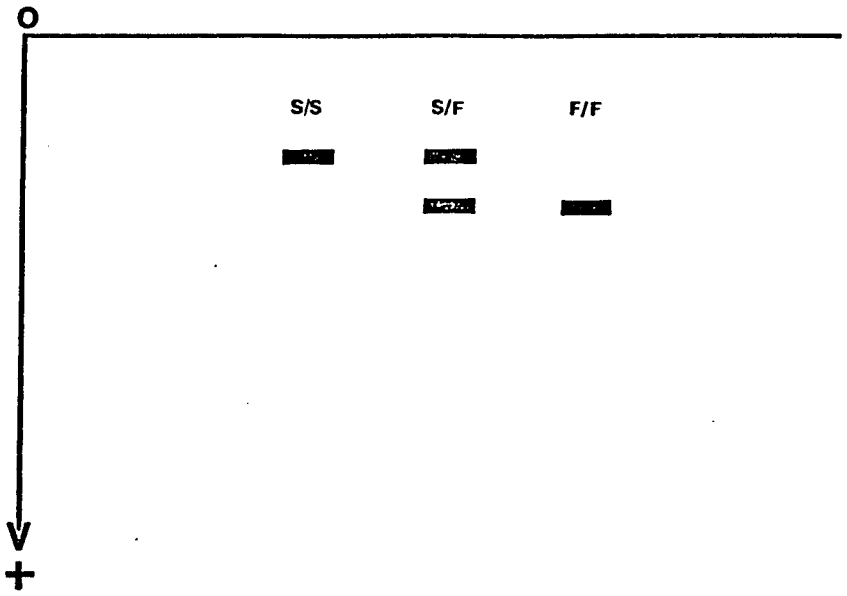


TABLE 15

A Chi-Square Comparison Of Observed LAP Allele Frequencies With Expected Values Derived From The Hardy-Weinberg Model - Blue Hill Falls

Season	X^2	DF	P
March	21.6	2	<.001
May	6.06	2	< .05
August	10.75	2	<.005
October	7.0	2	< .01

TABLE 16

A Chi-Square Comparison Of Observed LAP Allele Frequencies With Expected Values Derived From The Hardy-Weinberg Model: Pretty Marsh

Season	X^2	DF	P
March	2.13	2	< .50
May	5.76	2	< .10
August	15.6	2	< .001
October	4.3	2	< .25

the animals collected at Pretty Marsh survived the two week experiment, results are presented only for animals from Blue Hill Falls.

(1) Xanthine Dehydrogenase.

(Tables 16 and 17)

Electrophoretic analysis of caecal tissues of animals in both fed and fasted treatment groups shows the presence of two phenotypes in each group. These two phenotypes are the same as those noted earlier in Results (Section A2). One phenotype is characterized by a single band of Rf .36, while the second phenotype is characterized by having two bands: a band with Rf .36, and a second faster-moving band of Rf .40. Frequencies of the two phenotypes are shown in Table 16. Chi-square analyses (Table 17) shows no significant differences between treatment groups based on the phenotypic frequencies noted in Table 16.

(2) Malate Dehydrogenase.

(Figure 39, Table 17)

Electrophoretic analysis of caecal tissues of animals in both fed and fasted treatment groups indicates the presence in each group of three MDH phenotypes (Figure 39). These are the 2, 3A, and 3B phenotypes noted earlier in Results (Section A3). A chi-square test based on the frequencies of these phenotypes shows no significant difference between treatments (Table 17).

(3) Alkaline Phosphatase.

(Figures 40 and 41, Table 17)

Electrophoretic analysis of caecal tissues of specimens in both fed and fasted treatment groups shows the fed group to be represented by four phenotypes and the fasted group to be represented by

TABLE 17

The Effects Of Feeding And Fasting On Xanthine Dehydrogenase Phenotypes

Treatment	N	Phenotype .36	Phenotype .36-.40
Fed	9	2	7
Fasted	9	2	7

FIGURE 39

The effect of feeding and fasting on cytoplasmic malate dehydrogenase phenotypes in animals from the salt pond at Blue Hill Falls.

FEEDING – FASTING EXPERIMENT

MDH PHENOTYPES

BLUE HILL

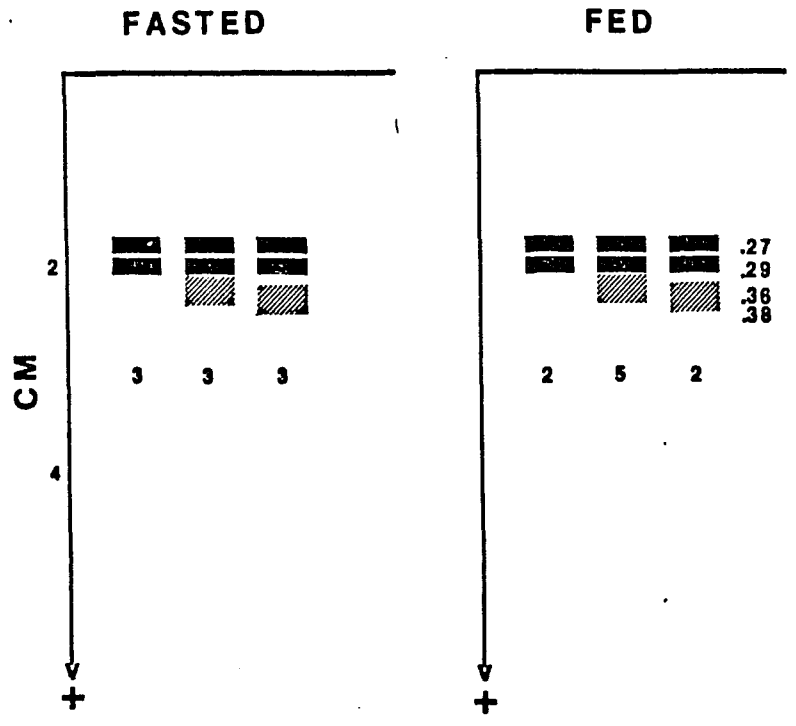


TABLE 18

The Effect Of Feeding And Fasting Of Isozyme Phenotypes: A Summary Of Chi-Square Analysis

Enzyme System	X^2	DF	P
Xanthine Dehydrogenase	0	1	.999
Malate Dehydrogenase	.925	2	< .75
Alkaline Phosphatase	7.2	1	< .01
Esterase	5	1	< .05

three phenotypes (Figures 40 and 41). In the fed group, the most frequent phenotype is comprised of four bands of Rf values .18, .33, .42, and .51. In the fasted group, the most frequent phenotype is comprised of two bands, having Rf values of .42 and .51, respectively. When the two treatment groups are compared by a chi-square test, on the basis of the frequencies of these two phenotypes, a significant difference between the treatment groups is obtained (Table 17).

(4) Acid Phosphatase.

Electrophoretic analysis for acid phosphatase did not yield analyzable results.

(5) Non-Specific Esterases.

(Figures 42 and 43)

Electrophoretic analysis of caecal tissues of specimens in both fed and fasted treatment groups demonstrated the existence of two phenotypes in the fed group and eight phenotypes in the fasted group (Figures 42 and 43). In the fed group, the most frequent phenotype, represented by eight individuals, is characterized by having three bands, of Rf values .45, .57, and .64 (Group I). The least frequent phenotype, represented by one individual, is characterized by four bands of Rf values .45, .57, .64, and .68. The bands of Rf .64 and .68 are characteristic of Group II isozymes. In the fasted treatment group, only one individual displays the three banded complex characteristic of Group I isozymes, while four individuals each possess bands of Rf .64 and .68 (Group II). Chi-square analysis of the fed and fasted treatment groups with respect to the relative frequencies of Group I and Group II band complexes shows a significant difference between the treatments (Table 17).

FIGURE 40

The effects of feeding and fasting on alkaline phosphatase phenotypes of animals from the salt pond at Blue Hill Falls. Fasting seems to result in the elimination of isozymes of low mobility.

FEEDING – FASTING EXPERIMENT

ALKALINE PHOSPHATASE PHENOTYPES

BLUE HILL

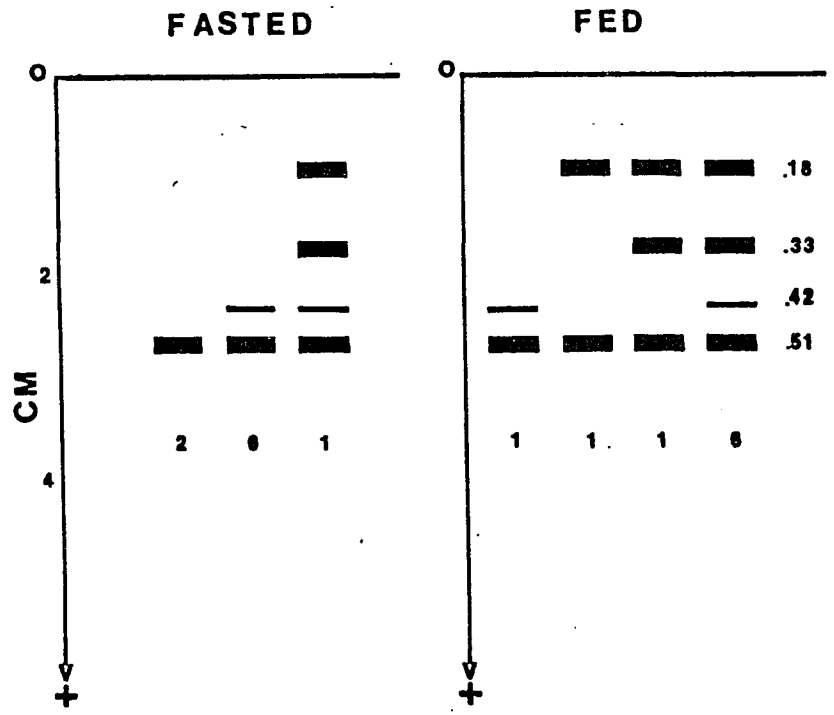


FIGURE 41

A photograph of a polyacrylamide gel showing the effects of feeding and fasting on alkaline phosphatase phenotypes.

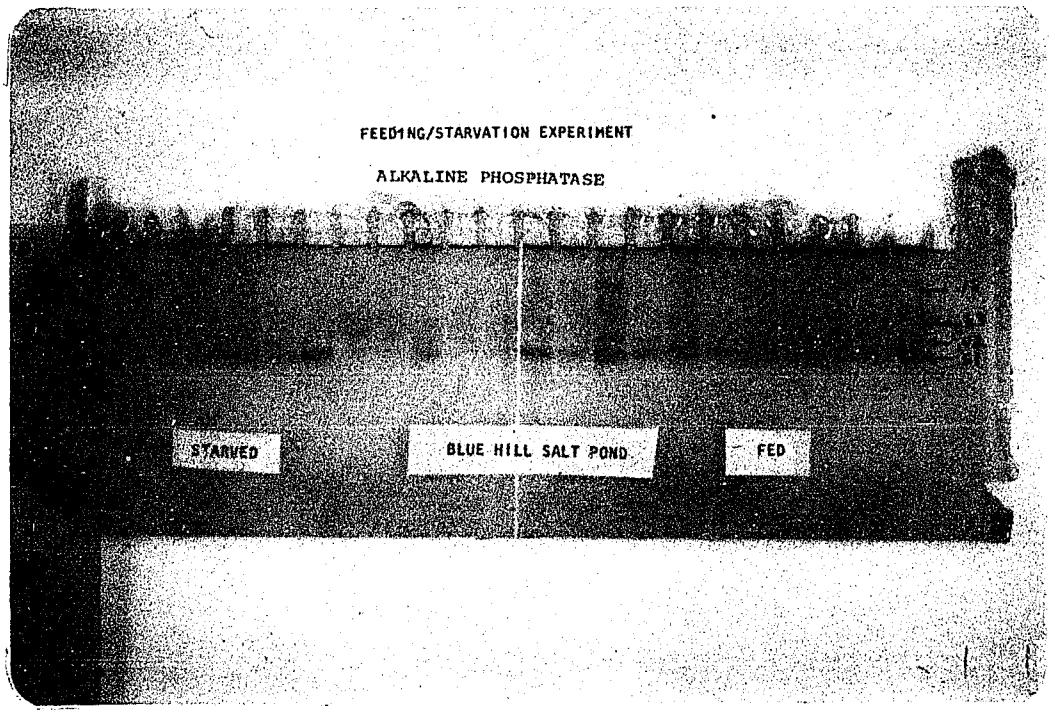

FEEDING/STARVATION EXPERIMENT
ALKALINE PHOSPHATASE
STARVED
BLUE HILL SALT POND
FED

FIGURE 42

The effects of feeding and fasting on esterase phenotypes of animals from the salt pond at Blue Hill Falls. Fasting seems to result in the manifestation of an isozyme band with an Rf of .68.

FEEDING–FASTING EXPERIMENT

BLUE HILL ESTERASE PHENOTYPES

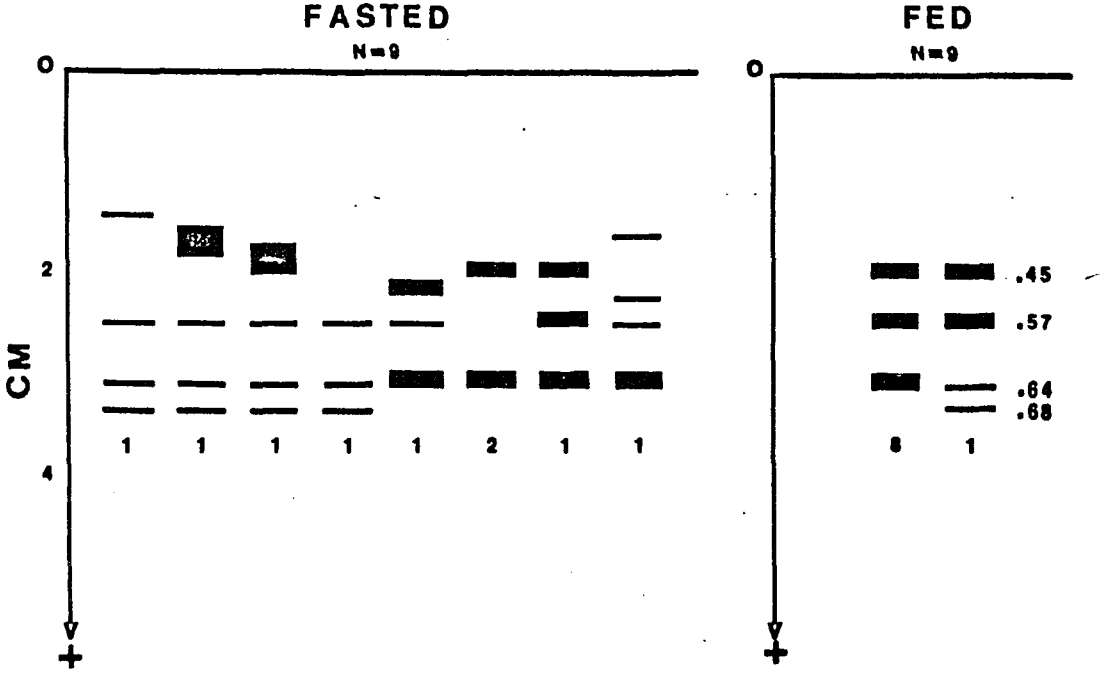
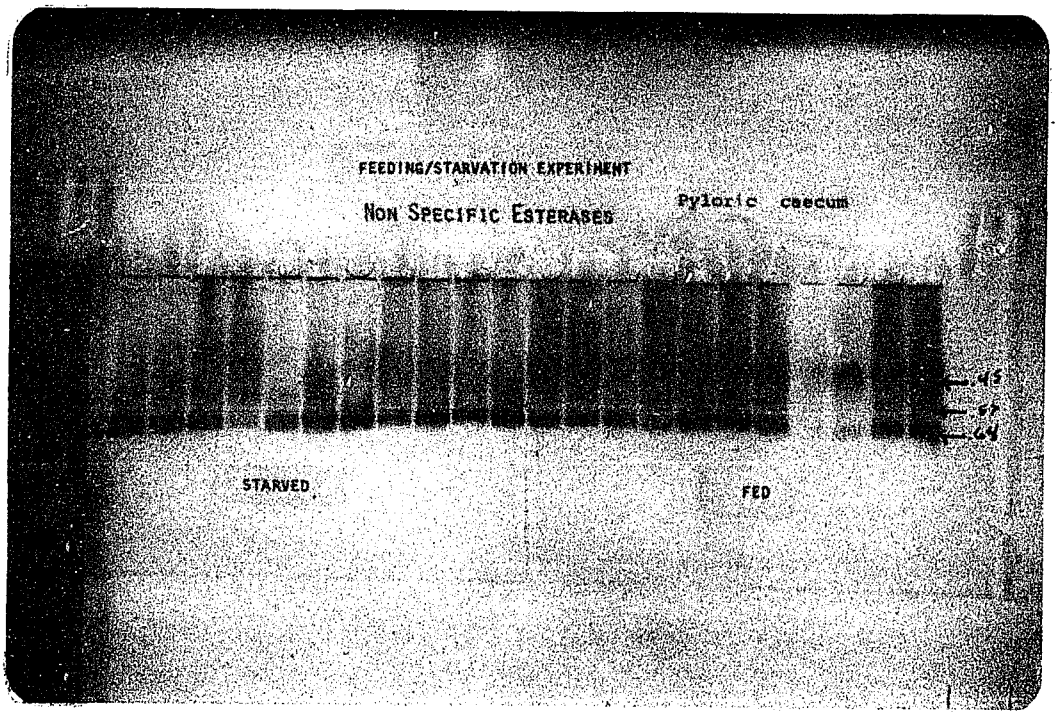


FIGURE 43

A photograph of a polyacrylamide gel showing the effects of feeding and fasting on esterase phenotypes. Animals are from the salt pond at Blue Hill Falls.


FEEDING/STARVATION EXPERIMENT
NON SPECIFIC ESTERASES
Pyloric caecum
45
67
44
STARVED
FED

DISCUSSION

A. Variation Among Geographically Separated Populations

(1) General Patterns Of Variation Related To Geography.

This project was initiated to search for geographically-related patterns of variation in certain morphological and biochemical characters of the sea-star Asterias forbesi. Expected patterns of variation, derived primarily from terrestrial examples, are clines, peripheral isolates, hybrid zones, or a combination of all three (Mayr, 1975).

A cline is defined as the progressive change in a character or characters, throughout a series of contiguous populations (Mayr, 1975). Such a pattern of variation is usually associated with gradients in environmental factors such as temperature, moisture, and salinity. Genetically, clines may result from two opposing forces, natural selection which tends to make populations different, and gene flow among populations which tends to make neighboring populations similar.

A second general pattern of geographic variation involves populations somehow isolated from the major distributional body of the species. As a result of a combination of such factors as selection, genetic drift, founder effects and imbreeding, isolated populations may show significant differentiation relative to nearby populations. Moreover, these processes may result in populations in which intraspecific variation is reduced, compared to non-

isolated populations of the same species (Dobzhansky et al., 1963; Mayr, 1975).

Hybrid zones may be defined as areas of increased variability, as compared to levels of variation in nearby populations. There are two general types of hybrid zones. In the first type, reproductive isolating mechanisms are incomplete in members of contiguous populations, such that some interbreeding may take place between these populations. In the second case, differentiation between populations has proceeded to the point where reproductive isolation is complete. However, secondary contact of members of these reproductively isolated populations may result in the breakdown of isolating mechanisms, resulting in a hybrid zone.

(2) Geographically-related Patterns Of Variation Expected In This Study.

The wide range of ecological conditions which exist within the transect used in making collections for this study, plus the sympatric relationship of Asterias forbesi and Asterias vulgaris suggest the possible existence of clines, peripheral isolates, and hybrid zones throughout the range of Asterias forbesi. Clines are expected to be associated with current-related gradients, like those which exist in coastal waters south of Cape Cod and all along the coast to Cape Hatteras. The geologically incised coastline and resulting spatial heterogeneity in the Gulf of Maine result in populations which are physically isolated from gene exchange with nearby populations. The population of animals in the salt pond at Blue Hill Falls approximates this condition of isolation. The possible distributional discontinuity and potent-

ially reduced gene flow between populations in the Gulf of Maine and those in the Gulf of St. Lawrence could result in significant differentiation in morphological and biochemical characters. However, the similarities in ecological features of Merigomish Beach and the Long Island Sound and Cape Cod regions permits one to test the hypothesis that similar ecological conditions in geographically remote areas result in the selection of similar genotypes and phenotypes.

(3) Morphological Variation In Skeletal Mass.

Comparison of populations examined in this study, based on the mean weights of carinal ossicles indicates that there is significant differentiation among populations. Moreover, a significant negative correlation of ossicle weights with latitude provide some evidence of clinal variation. The nature of this cline is not a perfect progressional change in ossicle weights from southern populations to northern populations in the transect investigated. Animals at Cape Cod and more southern sites have heavier, more massive skeletons, while those north of the Cape have lighter skeletons. Such a cline is not a gradual progression but is more like the "stepped" cline noted by Endler (1977), with the major step located near Cape Cod. Specimens from Blue Hill Falls show low mean weights of their carinal ossicles and a narrow range of variability for the particular latitudinal position from which they come. This may be at least partially attributable to the isolated nature of the salt pond. The mean weights of carinal ossicles from animals at Merigomish is somewhat greater than those of specimens from the Gulf of Maine and the range of variability more like

those of specimen collected from south of Cape Cod. Lowest weights of carinal ossicles were observed in animals from St. Ann's Bay, where it is possible that climatic influences are more influential than genetic factors. These climatic factors are long cold winters, which result in a shorter growth period, and low mean annual water temperatures.

A character like skeletal mass is probably both genetically determined and also a function of the environmental factors which affect growth. Growth, in turn, is determined by such factors as sea-water temperature, length of seasonally related feeding periods, and the availability of food. In a laboratory based study of feeding rates of Asterias forbesi on oysters in Long Island Sound, MacKenzie (1969) showed that Asterias forbesi displays a maximum feeding rate at about 20°C. He found that animals are reluctant to feed at temperatures much above 20°C and below about 5°C. Using the idea that temperature is important in governing feeding behavior and that the temperatures at which feeding occurs are time-limited, it is possible to construct a simple definition of foraging season. The foraging season is the number of months when the mean monthly temperature at each location falls within MacKenzie's recorded range of preferred feeding temperatures (5°C - 20°C). Growth, then, is possibly related to foraging season. Mean weights of carinal ossicles at each location correlate significantly ($r = .66$) with foraging season, shown in Figure 1. The definition of foraging season for this assumes that food is equally available at all locations and that no adaptation in feeding behavior has occurred. While the positive correlation of carinal ossicles

weights with foraging time suggests a relationship between ossicle weights and environmental factors which affect growth, other evidence suggests that this relationship is not so clear cut. For example, if weights of carinal ossicles were strongly determined by growth, then some correlation of weights of carinal ossicles with size of the animal should exist. Small animals would be expected to have light skeletons, and large animals would be expected to have heavy skeletons. When all animals from all populations examined are analyzed, this does not seem to be the case. When animal size, expressed as R in millimeters, is compared with weights of carinal ossicles, a weak correlation is obtained ($r = .49$).

An alternative explanation to that just mentioned depends on some level of genetic determination of skeletal mass. Under this model, population differences might be explained in terms of evolutionary processes such as natural selection, gene flow, genetic drift, founder effect, and hybridization. The evidence for an immediate genetic basis which determines skeletal mass is not strong, and is circumstantial in nature. This evidence is derived from observations made on animals collected from the salt pond at Blue Hill Falls. First, the low mean carinal ossicle weight and the narrow range of ossicle weights associated with that low mean weight, suggest directional selection of animals with light skeletons. Second, the mean carinal ossicle weights and ranges around those means for Asterias forbesi and Asterias vulgaris are almost identical. This similarity in skeletal mass suggests hybridization. There are thus three types of Asterias in the salt pond: Asterias vulgaris, Asterias forbesi, and light-skeletoned forbesi-like

animals. These atypical animals in the salt pond have characteristics of both Asterias forbesi and Asterias vulgaris. These forbesi-like sea-stars have pigmentation and major pedicellaria similar to those of typical Asterias forbesi, but have skeletons which in terms of skeletal mass better fit the description of Asterias vulgaris. The possession of a vulgaris-like skeleton suggests the introduction and selection for genes from Asterias vulgaris into the Asterias forbesi genome. The extent of possible genic introgression at each location may be estimated by enumerating the individuals which have carinal ossicle weights that fall within the range of variation of the Asterias forbesi-like animals from Blue Hill Falls. Such a comparison is shown in Table 18, and indicate that introgression is low to the south and higher to the north of Cape Cod. Introgression is maximal in the animals at Blue Hill Falls, but is not complete; that is, there are still typical Asterias forbesi identifiable in the salt pond. This observation is confirmed by Vadas (1981).

Differences noted in carinal ossicle weights between populations could be explained in terms of the effects of natural selection on a common genetic stock. In particular, a wide range of variability is postulated to exist in Asterias forbesi. Diversifying selection associated with the differences seen between the Virginian and Boreal regions could account for the sharp differences observed in the mass of skeletons south and north of Cape Cod. Similarities in ranges of variation in the weights of ossicles from specimens from Merigomish and the Long Island and Cape Cod regions could be accounted for in terms of similarities of selection pressures associated with environmental similarities.

TABLE 19

The Percentages Of Samples At Each Location Which Fall Within The Range Of Variation Noted At Blue Hill Falls.

Location	N	Percentage - %
Beaufort	35	11 %
Long Island	50	0 %
Cape Cod	50	2 %
Blue Hill Falls	50	82 %
Pretty Marsh	50	52 %
Merigomish	50	48 %
St. Ann's Bay	50	18 %

Gene exchange between populations could be accomplished by migration of adults, rafting of juveniles on debris or more likely by larval transport. It seems reasonable to suppose that the possibilities for gene exchange among populations south of Cape Cod is potentially great. If this is so, and populations are differentiated from each other, as seems to be the case, then it would seem that the selection pressures at Beaufort, Long Island and Cape Cod must be rather similar. The differences in skeletal mass of animals between locations south of Cape Cod and the Gulf of Maine suggest that Cape Cod may serve as a filter to gene exchange. If selection pressure is high, then a small amount of gene exchange would not retard differentiation. The differences in weights of carinal ossicles of specimens collected at Pretty Marsh and Blue Hill Falls may be attributable to reduced gene exchange between these two physically isolated populations. As will be noted later, environmental conditions at Pretty Marsh and Blue Hill Falls are quite different and could provide different selection pressures. Moreover, genetic drift and the founder principle are likely to be operational in the salt pond populations. It is possible that larvae from south of Cape Cod are transported via the Gulf Stream to the more northern locations. Such transport could account for the similarity of ranges of weights of ossicles from specimen collected at Merigomish Beach and the Long Island region. However, it is more likely that the cold waters of the southerly flowing Labrador current act as an effective barrier to larvae entering the Gulf of St. Lawrence via this route. The similarities of ossicle weights of animals from Merigomish and Long Island are then most likely

attributable to similarities in selective pressures, resulting from similarities of the two environments. While gene exchange between Merigomish and St. Ann's Bay is possible as a result of currents that flow out of the Gulf of St. Lawrence and then southwest around Cape Breton, selection may in part account for the differences between the two populations.

The selective advantage of regional differences in the weights of ossicles is speculative. It is possible that the heavier skeletons help to protect animals in southern locations from predation by an assemblage of predators including fish, gulls, and arthropods such as lobsters (Galtsoff and Loosanoff, 1939; Ernst, 1963). Given the apparent reduced effectiveness of tube feet on unconsolidated substrates such as those found south of Cape Cod, a heavier more massive skeleton could be of advantage in preventing displacement by storm surge. Predation on Asterias forbesi with heavier skeletons could be energetically more expensive than predation on animals with lighter skeletons. As a result, a predator could be at a disadvantage from two stand points. First, relatively more energy must be expended in penetrating the skeleton, and second, less energy is acquired from animals with heavier skeletons. Animals with lighter skeletons may have more organic material per unit of total body weight than do animals with more massive skeletons. Finally, animals with a heavier skeleton may be better predators. In northern populations, a lighter skeleton could prove to be advantageous in two ways. First, a thinner, more flexible skeleton could allow animals to fit into small crevices and hiding places, giving protection from predators and ice scour in winter. Second,

tube feet would seem to be a more effective anchoring device than is a heavy skeleton, on the harder substrate found in more northerly locations. Thus, a heavier skeleton in northerly locations may be less advantagous in protecting against storm surge, and could be of a disadvantage in terms of the energy costs necessary to maintain it.

(4) Biochemical Variation - Background Information.

Evolutionary processes have resulted in two basic ways of increasing metabolic flexibility and diversity in enzyme systems (Powell, 1976). One is to maintain multiple alleles at a structural locus coding for a given enzyme. The single locus with two codominant alleles is a simple example. Another strategy is to maintain multiple isozymal loci. In the latter case, a simple genome has several loci each of which produces an enzyme product with similar functions. These different loci might then become specialized for optimal functioning under different conditions (Powell, 1976).

These two strategies should not be regarded as mutually exclusive, since different evolutionary lines may exhibit varying degrees of both. In a comparison of invertebrates with vertebrates, it is clear that the former diversify their metabolic functions more often by polymorphism at a given locus than by increasing the total number of loci. On the other hand, in the vertebrates, there exist many multiple locus isozymes. In contrast to other echinoderms, the sea-star Asterias forbesi has low levels of genetic polymorphism. Schopf and Murphy (1973) indicate an average heterozygasity of 2.1%, which is low compared to levels of about

17% for other echinoderms (Table 19). This evidence suggests that this sea-star has opted for the multi-locus strategy.

It appears that different enzymes tend to have different levels of genetic variation which transcend taxonomic lines. For example, evidence suggests that the non-specific esterases are polymorphic in all taxa (Powell, 1976). Perhaps levels of genetic polymorphism are related to metabolic function rather than to more specific taxonomic categories.

Johnson (1974) has divided enzymes into the following three classes:

(a) Variable substrate enzymes: Enzymes that act on variety of substrates originating outside the organism, and varying in concentration. These enzymes display high levels of genetic variability. Examples are the esterases and the phosphatases.

(b) Regulatory enzymes: Enzmyes that regulate the flow of metabolites along metabolic pathways, and are important in determining reproductive fitness by catalyzing more sensitive steps in a pathway on which natural selection might operate. In Drosophila, small vertebrates and man, these enzymes are less variable than those noted in group (a). An example is xanthine dehydrogenase.

(c) Non-regulatory enzymes: Enzymes that show equilibrium conditions with regard to substrate/product ratio. These enzymes are the least variable of the three types. Examples are malate dehydrogenase and glutamate oxaloacetate transaminase.

The biochemical aspects of the present study on geographically-related variation in Asterias forbesi includes enzymes from all three of these categories. Whether levels of variability correspond to

TABLE 20

Genetic Variation In Echinoderms Expressed As Average Heterozygosity Per Locus

Animal	Reference	No. of Loci	Heterozygosity
Cucumaria curata	Rutherford, 1977	12	0
Asterias vulgaris	Schopf and Murphy, 1973	26	.011
Asterias forbesi	Schopf and Murphy, 1973	27	.021
Asterina spp.	Matsuoka, 1981	13-16	.037
Arbacia punctulata	Marcus, 1977	12	$.123 \pm .17$ --- $.184 \pm .26$
Ctenodiscus crispatus	Schick, Taylor, & Lamb, 1981	13	.174
Ophicmusium lymani	Ayala & Valentine, 1974	15	.17
Nearchaster aciculosus	Ayala & Valentine, 1974	--	.21
Non-Insect Invertebrates	Powell, 1976	$\sim$10	$.102 \pm .021$

those noted above, however, is not well known for Asterias forbesi.

(5) Biochemical Variation In Xanthine Dehydrogenase.

Xanthine dehydrogenase is represented by two electrophoretically detectable phenotypes whose relative frequencies vary with geographic location. It should be noted that the band of Rf .36 is found in all animals from all locations. It is the presence of the band with the Rf of .40 which serves to differentiate populations from one another.

Patterns of phenotypic variation are evident which correlate with broad patterns of environmental conditions in the various regions studied. A clinal shift in the frequency of the .36-.40 phenotype is most evident among those populations south of Cape Cod. Such a shift in frequency may correspond to a current related, gradient in sea-water temperature. North of Cape Cod, the frequency of the .36-.40 phenotype is highest in the Blue Hill salt pond, contrasting with the rather low frequency at Pretty Marsh. This large and abrupt frequency difference within a short distance appears to be related to the degree of isolation and to the contrasting ecological conditions which exist in the two locations, especially in the ranges of seasonal sea-water temperature.

The frequency of the .36-.40 phenotype rises from about 40% at Pretty Marsh to 56% at Merigomish and at St. Ann's Bay. The increase between Pretty Marsh and Merigomish may be attributable to contrasting environmental conditions mentioned earlier. Moreover, the frequency at Merigomish approaches that of a theoretical location somewhat south of Long Island Sound. The identical frequencies at Merigomish and St. Ann's Bay may result from chance, or possibly

from larval exchange driven by water currents flowing from the Gulf of St. Lawrence around Cape Breton and southwest along the coast of Nova Scotia. While no quantitative data concerning the effects of selection pressure is available, based on the two XDH loci observed in most areas, it appears that selection could be an effective force in differentiating populations of Asterias forbesi. Except at the two Canadian locations, gene flow seems to be less effective in maintaining genetic integrity.

Establishing relationships between geographically related, environmental parameters and patterns of isozyme variability help to indicate the adaptive significance of the isozymes considered. Temperature may be regarded as one of the most influential agents of natural selection that may affect the genetic structure of populations of marine organisms. Temperature has been shown to be of significance in maintaining genetic variability in populations of the ectoproct Schizoporella unicornis (Schopf and Gooch, 1970), the clam Mercenaria mercenaria (Pesch, 1974), the sea urchin Arabacia punctulata (Marcus, 1977), and the sea anemone Metridium senile (Hoffman, 1980). It is therefore likely that in Asterias forbesi isozymal variability is somehow related to locational differences in sea-water temperature. It is possible that variability in the frequencies of the XDH phenotype is related to mean annual water temperature at each location. Mean annual sea-water temperatures are relatively high at Beaufort, North Carolina, and decrease progressively as one goes northward to St. Ann's Bay. This gradient in mean annual temperature could be reflected in a cline in XDH phenotype which relate to those temperatures. A second possible way that XDH phenotypes and temperature could be related involves

differences in seasonal ranges of sea-water temperatures at each location. It could be that XDH phenotypic variability somehow relates to temperature range rather than an annual mean. Finally, enzyme variability may be related to an optimum or critical water temperature, or both.

No significant correlation ($r = -.3$) exists between the frequency of the .36-.40 XDH phenotype and mean annual temperature at each location. The frequency of the .36-.40 phenotype does show a strong correlation ($r = .89$) with a mean annual temperature range at each location. Thus, in those areas where the range is comparatively narrow, such as Beaufort, North Carolina and Pretty Marsh, Maine, the frequency of the .36-.40 phenotype is low. In those areas where the range is greater, such as Long Island, Cape Cod, and Blue Hill, the frequencies are greater. The progressive increase in the magnitude of the ranges thus accounts for the clinal shift from Cape Cod. It may be that specific temperatures are critical to determining the frequency of isozymal bands. The frequency of the .40 band is negatively correlated (Figure 44) with deviations of maximum mean monthly temperatures from $20^{o}C$. Thus, in those areas where the maximum deviation from $20^{o}C$ is greatest, such as Beaufort and Pretty Marsh, the frequency of the .40 band is low. Likewise, in those areas where the maximum mean monthly temperature approaches $20^{o}C$, the frequency of the .40 band is high. It seems probable that a temperature of $20^{o}C$ is critical to the relative frequency of the .40 band.

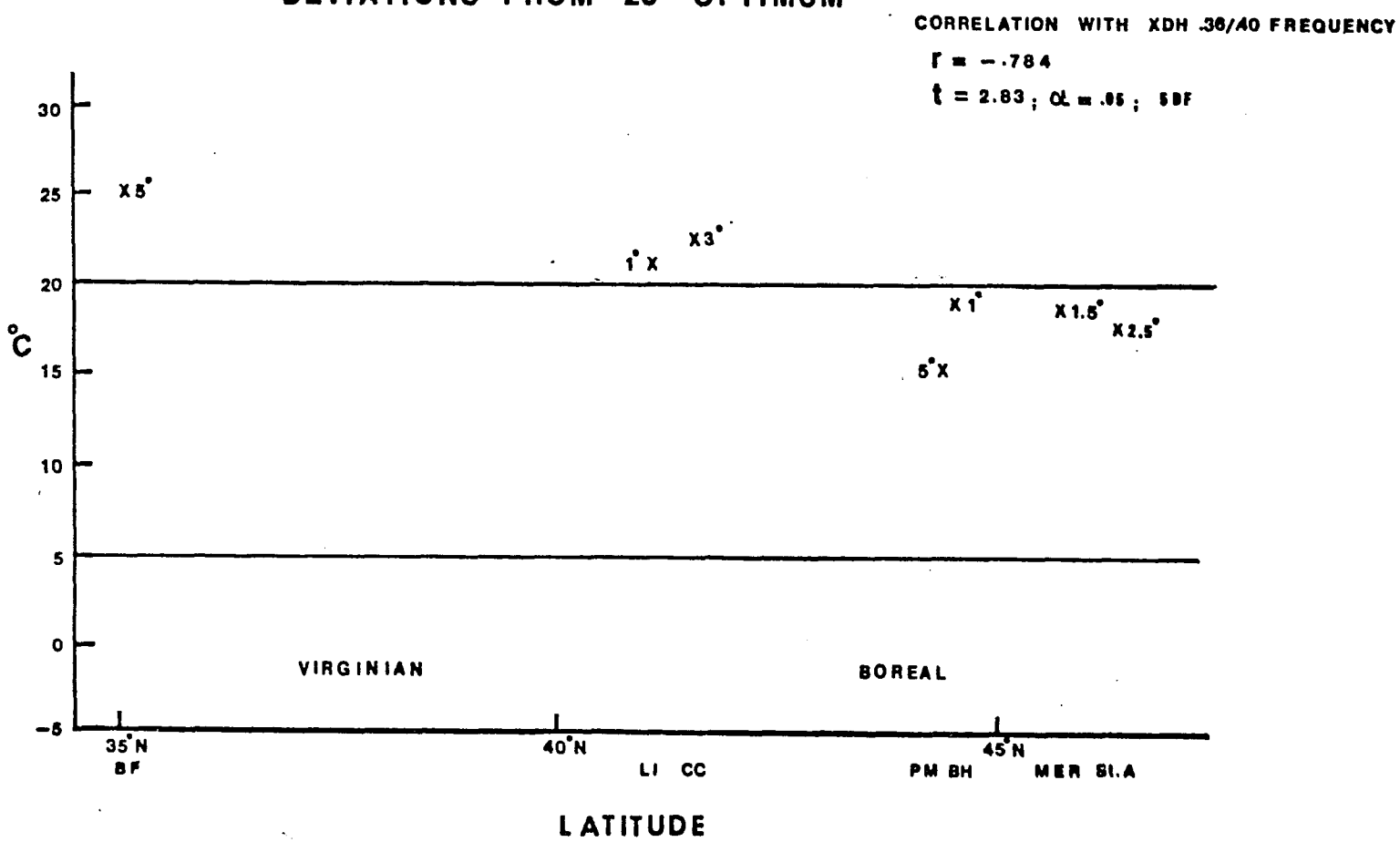
Two slightly different approaches to the question of adaptive significance of temperature-related isozyme variability have been

FIGURE 44

Deviations of monthly, mean, maximum water temperatures from $20^{o}C$, at each sampling location. Sources of information are the same as those noted in Figure 1.

MAXIMUM MEAN WATER TEMPERATURES

DEVIATIONS FROM 20° OPTIMUM



presented by Koehn (1969) and by Hochachka and Somero (1968). Koehn was able to demonstrate the existence of two different alleles at the Est I locus in the Gila mountain sucker Catastoma clarkii. These alleles varied in frequency over a north-south cline. The activity (Vmax) of the more frequent allele in southern populations increased as temperature increased from 0^{o} to $37^{o}C$, while activity of the more frequent allele in northern populations increased as temperature decreased. The implication is that selection is acting on the catalytic activity of the enzymes produced by these two different alleles. Alternately, Hochachka and Somero have suggested that selection may be operating on kinetic properties of isozymes other than catalytic activity. They were able to show in a study of LDH isozymes in fish, that enzyme affinity for its substrate expressed as the inverse of Km, is more sensitive to natural selection, than is molecular activity.

It is postulated that the two XDH isozymes of Asterias forbesi are temperature adaptive, and function better at different temperatures. The isozyme of Rf .36 may function at lower temperatures, while the isozyme of Rf .40 may function at higher temperatures. Animals with the single Rf .36 form of the enzyme are restricted to locations where temperature ranges are relatively narrow, while those animals with both Rf .36 and Rf .40 forms of the enzyme possess biochemical flexibility which allows them to survive in areas where temperature ranges are wider.

As previously noted, some evidence exists for the association of specific bands with temperature. Specifically, this evidence includes (1) the association of the .40 band with a critical temperature

of 20°C. That 20°C is biologically important to Asterias forbesi is confirmed by Franz, Worley, and Merrill (1981) who found the biogeographic distribution of Asterias forbesi to be limited by sea-water temperatures of 20°C, and by MacKenzie (1969) who noted maximum feeding rate in Asterias forbesi at about 20°C, (2) the relatively high frequency of the .36 phenotype at Pretty Marsh where the seasonal temperature range is about 0° to 15°C and the mean annual temperature about 7°C, and (3) the occurrance of the .36 locus in Asterias vulgaris, the cold water cogener of Asterias forbesi. A limited comparison (30 specimens) of XDH phenotypes of Asterias forbesi and Asterias vulgaris from Blue Hill Falls and from the New Hampshire coast indicate that only one XDH locus is present in Asterias vulgaris. The enzyme associated with this locus has an Rf of .36. The presumed adaptive significance of the XDH phenotypes can be explained in terms of geographic speciation noted by Schopf and Murphy (1973). They suggest that Asterias forbesi and Asterias vulgaris evolved from a Miocene or early Pleistocene ancestor which ranged freely both north and south of present Cape Cod. Franz, Worley, and Merrill (1981) have suggested that this ancestor may have arrived on the eastern coast of the United States by trans-arctic migration from northern pacific waters. The ancestor was presumably adapted to life in cold waters. The subsequent elevation of Cape Cod and George's Bank could have created a barrier dividing the ancestral Asterias into at least two sub populations, including a northern population which under the influence of cold Labrador currents differentiated into Asterias vulgaris, and a southern population, which under the influence of warm Gulf Stream currents different-

iated into Asterias forbesi. It is suggested that the wide ranging ancestral Asterias possessed the gene associated with the .36 XDH enzyme and was adapted to cooler waters associated with glacial periods. The .36 band was retained in Asterias vulgaris as an adaptation to cooler waters. Under the influence of the wider temperature ranges resulting from the Gulf Stream and in the population south of Cape Cod, the gene associated with the band of Rf .40 could have evolved by gene duplication, and subsequent specialization by mutation. Finally, it is impossible to say whether selection pressure on Asterias forbesi is acting on molecular activity or on some other kinetic property such as enzyme-substrate affinity.

(6) Biochemical Variation In Malate Dehydrogenase.

Cytoplasmic malate dehydrogenase is represented by six bands of individually varying mobilities which are combined to give a total of seven different phenotypes (Figure 9). The relative frequencies of these phenotypes vary with latitude and mean annual sea-water temperature.

The interpretation of geographic variation in malate dehydrogenase is difficult for four reasons. First, the large number of bands and resulting band combinations is somewhat confusing, in attempting to find patterns of variation. Second, differences in staining intensities in certain bands, notably the faster moving ones of Rfs .42 and .48 make interpretation difficult. Third, inconsistencies in the mobilities of the faster moving bands, again of Rfs .42 and .48, make interpretation somewhat uncertain. A specific example of this is the situation where a number of individuals possessing these particular bands are analyzed electrophoretically on the same gel, presumably

under the same experimental conditions. While all the individuals show the presence of the bands, the mobilities of the bands from individual to individual are not precisely the same. The slight mobility differences do not seem to show any consistent patterns at all. Fourth, interpretation of MDH is difficult because of problems with statistical analysis noted in Results.

If Results for MDH are treated in the way that Schopf and Murphy (1973) suggest, then only the bands of Rfs .27 and .29 are interpretable. Since each band is regarded as representing a monomorphic locus, no variation exists within or between populations. The absence of polymorphism is consistent with low levels of genetic variation observed for other regulatory enzymes (Johnson, 1974; Powell, 1976).

Despite the problems noted previously, three different patterns of variation are evident which relate MDH phenotypes to geographic location. One patterns involving all phenotypes and all locations indicate that populations of Asterias forbesi are divisible into three assemblages of organisms (Figure 11). The southern-most assemblage at Beaufort, North Carolina is represented by only the 2 and 3A phenotypes. The 3A phenotype is most frequent. Likewise, the northern most assemblage at St. Ann's Bay is represented by the 2 and 3A phenotypes. Unlike Beaufort, the 2 phenotype predominates. It may be that extreme climatic conditions are responsible for the absence of more anodally moving bands. The larger mid-range assemblage involves all phenotypes. While a number of different explanations of these facts are possible, a simple genetic interpretation assumes that each band represents a separate monomorphic locus. If this is

the case, then the 2, 3A, 3B, 3C and 3D phenotypes can be regarded as homozygotes. All possible crosses between these homozygotes results in ten kinds of heterozygotes, but phenotypically only six can be identified. Only two of these heterozygote classes, 4A and 4B (Figure 9) are observed in nature and are present in low frequency (Table 5). The heterozygotes are found only in the mid-range assemblage and are not found at Beaufort and St. Ann's Bay. As noted earlier in Results, no chi-square analysis was performed due to problems associated with the calculation of expected values.

A second pattern of variation involves populations which are somewhat unique because of their geographic locations and their particular ecological features. The ecological similarity of the Long Island Sound - Cape Cod region and Merigomish has been noted previously. This similarity is reflected in the presence of particular MDH phenotypes found at Long Island, Cape Cod, and Merigomish only. These MDH phenotypes are those designated 3C and 3D (Figure 9; Table 5). They are not present in animals collected at Beaufort, Blue Hill Falls, Pretty Marsh, or St. Ann's Bay.

The contrasting environments of Blue Hill Falls and Pretty Marsh show differences in the frequencies of both 2 and 3A phenotypes. At Blue Hill Falls, the frequency of the 2 band phenotype is 52% while at Pretty Marsh the frequency is 42%. At Blue Hill Falls, the frequency of the 3A phenotype is 30% while at Pretty Marsh it is 46%. Thus, differences in phenotypic frequencies exist in neighboring populations. These differences may be partially attributable to the enclosed nature of the pond and to related evolutionary processes such as selection, gene exchange, genetic drift, and founder effect.

A final pattern of variation which relates MDH phenotypes to geographic location is manifested as a cline which involves the 3A phenotype. The frequency of the 3A phenotype is highest at Beaufort, North Carolina and declines progressively northward over the collection transect, reaching a minimum at St. Ann's Bay. A strong positive correlation with mean annual temperature suggests its potential influence in determining the frequency of this MDH phenotype. The relationship of temperature and the frequency of the 3A phenotype is reinforced by the existence of a strong correlation of seasonal water temperature change with the frequency of blue 3A phenotype. This relationship is discussed in section B.

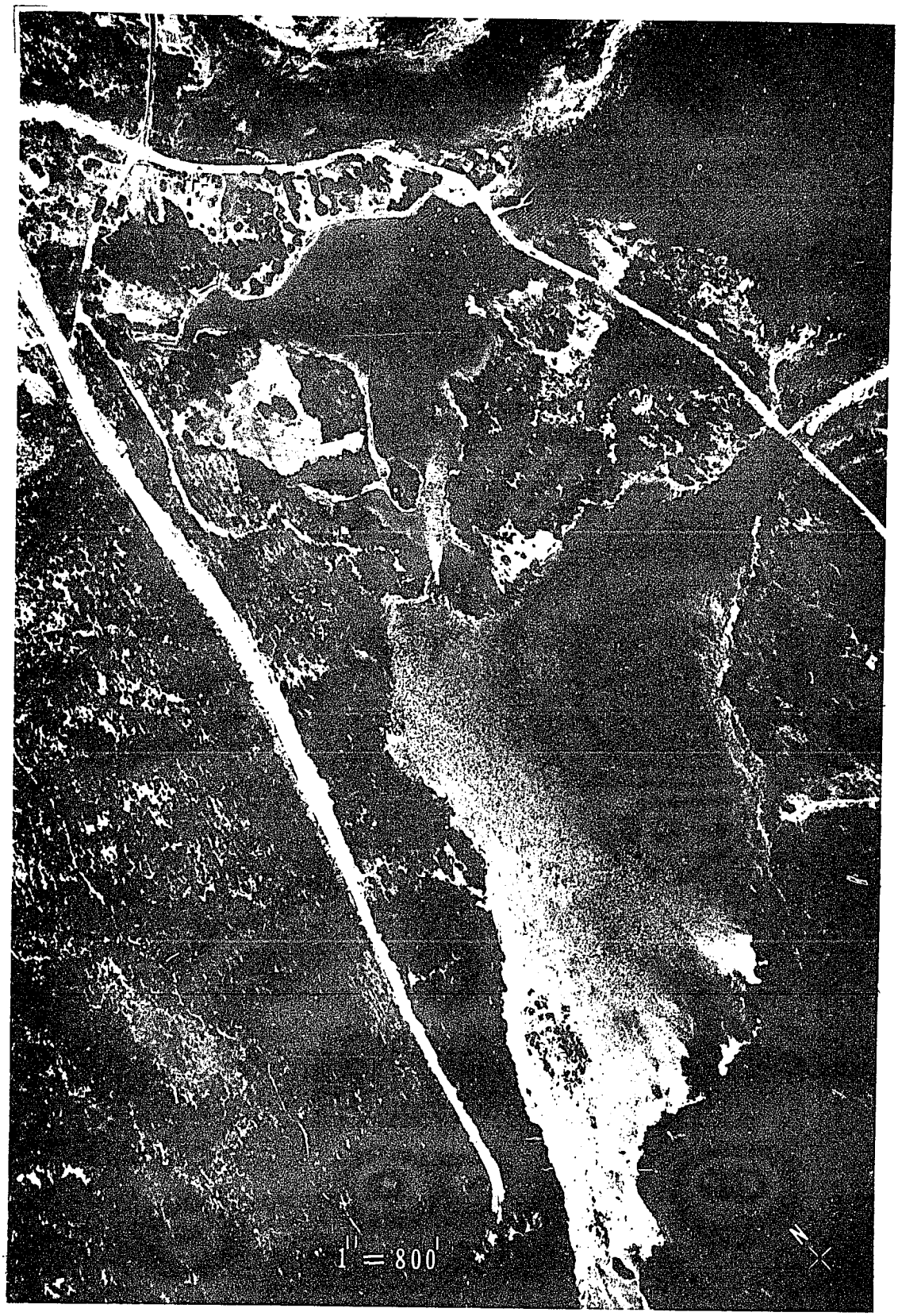
B. Seasonal Isozyme Variation At Blue Hill Falls And Pretty Marsh

(1) A Comparison Of Blue Hill Falls And Pretty Marsh.

A comparison of the salt pond at Blue Hill Falls with Pretty Marsh shows that the salt pond contains a unique population of Asterias forbesi. The uniqueness of this population is attributed in part to its physical isolation from Gulf of Maine influence (Figure 45). This physical isolation in all probability minimizes genetic exchange with nearby populations via adult migration and by larval transport, and favors such phenomena as genetic drift (Dobzhansky et al., 1963). Moreover, physical isolation creates locally atypical environmental conditions, which differ from ecological conditions in neighboring populations. For example, at the salt pond, seasonal temperature cycles include ranges of about -1^{o}C in February up to about 20^{o}C in late August. It may be that this range is even greater than as stated here. At Pretty Marsh, the

FIGURE 45

An aerial view of the salt pond at Blue Hill Falls. Animals were collected primarily from the area outlined with dotted line. The area of the salt pond is approximately 8.6 hectares and 5-15′ deep in the area where animals were collected.


1" = 800'
N

measured temperature range was of a lesser magnitude, from 0°C in February to 15°C in August, reflecting conditions more like those reported for the Gulf of Maine.

Seasonal salinity readings are somewhat different at Blue Hill Falls than they are at Pretty Marsh. During March and May at Blue Hill Falls, the salinity remained at 32 °/oo, rose to 34 °/oo in August, and dropped to 33 °/oo in October. At Pretty Marsh, salinity remained at 32 °/oo during March, May, and August, but rose to 33 °/oo in October.

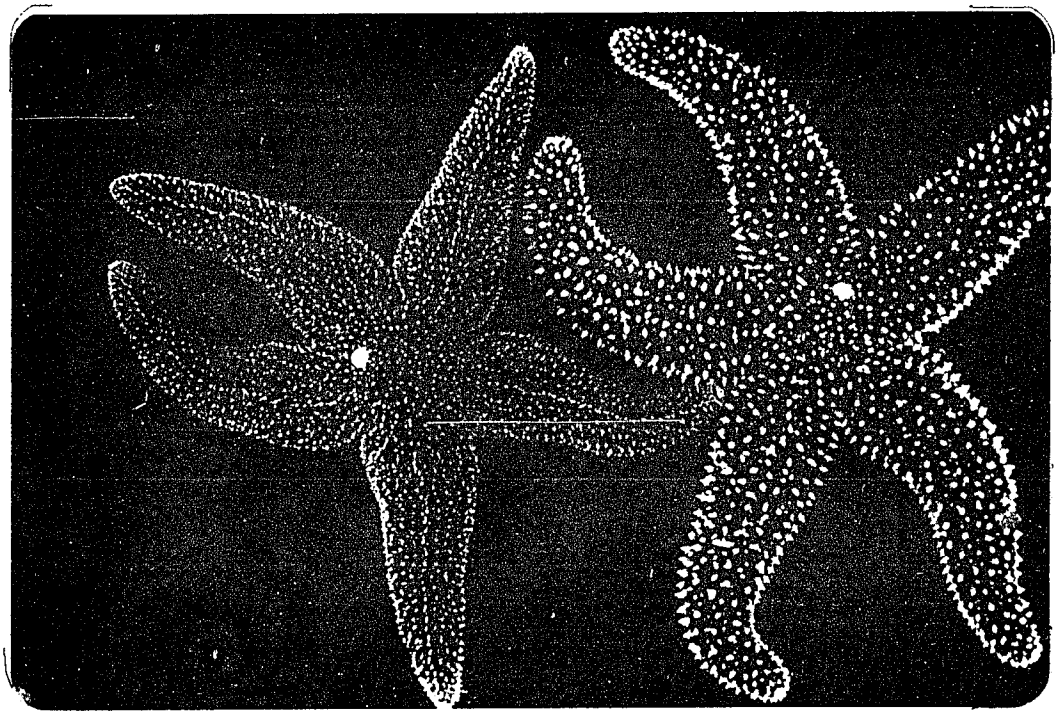
The estimated densities of Asterias forbesi are different at Blue Hill Falls and at Pretty Marsh. Asterias forbesi is extremely abundant at Blue Hill Falls, and is scarce at Pretty Marsh.

Nutritional resources are different at Blue Hill Falls and Pretty Marsh. Potential food sources such as bivalves, barnacles, and snails are extremely abundant at Blue Hill Falls and far less abundant at Pretty Marsh.

A comparison of animals in the salt pond population with those at Pretty Marsh demonstrates the possible effects of spatial heterogeneity on both morphological and biochemical characters. As has been previously noted, the forbesi-like animals in the salt pond display some unusual features. The taxonomic description of Asterias forbesi characterizes the skeleton as being heavy and massive. The forbesi-like animals in the salt pond have uniformly light, reticulate skeletons, which are almost identical to those of its congener, Asterias vulgaris (Figure 46). The genetic bases underlying ossicle weights and the similarity of the skeletons of Asterias forbesi and Asterias vulgaris from the salt pond are uncertain. At Pretty Marsh the mean weight of the carinal ossicles is significantly greater

FIGURE 46

Two specimens of Asterias forbesi. The more typical animal on the right is from Long Island Sound. The forbesi-like animal on the left is from the salt pond at Blue Hill Falls.



than the mean weight of ossicles of animals from the salt pond. Also, the range of variation of ossicles weights of animals from Pretty Marsh is greater than the range of carinal ossicle weights of animals from the salt pond.

Biochemically, the frequency of the .36-.40 XDH phenotype in salt pond animals is high, close to 90%, the highest found throughout the collection range. Only about 10% of the animals examined display a single band with an Rf of .86. By contrast, at Pretty Marsh, 7 miles away, the frequency of the .36-.40 phenotype is about 40%, one of the lowest frequencies for this phenotype found throughout the collection transect. Thus, it appears that the population of forbesi-like animals in the salt pond represents a population displaying some signs of reduced variability as has been noted for certain marginal populations of Drosophila pseudoobscura (Dobzhansky et al., 1963). A number of genetically related factors involving selection, reduced gene flow, founder effect, inbreeding, and genetic drift could explain this condition.

(2) Seasonal Cycles.

The ecological dissimilarity between Blue Hill Falls and Pretty Marsh and the differences of the animals in the respective locations suggest the possible existence of differences in seasonal cycles involved with feeding and reproduction. If disparities in seasonal cycles exist, then they may be reflected in seasonally related change in the frequencies of isozyme phenotypes, or possibly in the numbers of individuals displaying enzyme activity detectable by electrophoretic analysis.

Research on the natural history of Asterias forbesi done by Galtsoff and Loosanoff (1939) and MacKenzie (1969) has revealed

the existence of seasonally related patterns of behavior related to feeding and reproduction. In particular, MacKenzie (1969) has shown that Asterias forbesi in Long Island Sound displays two seasonal periods of maximal feeding activity and two periods of minimal feeding activity. Maximal consumption of oysters was observed in May-June, and October-November. Minimal consumption was noted in August and September, and again in January-February-March. Sherman (1966) in an investigation of the reproductive biology of selected echinoderms, reports on seasonally related change in hepatic indices in Asterias forbesi north of Cape Cod. She found the hepatic index to display two maxima, one in May-June and a second in late August-September. The index was low during periods from December to April, and from late June to late July. The coincidence of feeding periods with periods when the hepatic index is increasing suggests a direct effect of nutritional state on the size and development of the pyloric caeca in Asterias forbesi.

With the assumption that cycles somewhat similar to these exist in the forbesi-like animals from the salt pond at Blue Hill Falls and Pretty Marsh, it seems logical to suppose that if seasonally related isozyme variation exists, that it could very well relate to nutritional status as it is reflected in seasonally correlated feeding behavior, changes in hepatic indices and/or seasonal changes in biologically relevant environmental factors such as seawater temperature.

(3) Seasonally Related Isozymal Variation: Introductory Statement.

Seasonally-related isozymal variation is evident in certain enzyme systems in animals from both Blue Hill Falls and Pretty Marsh. Moreover, the two populations differ in the patterns of variation,

which are in turn related to locational differences in seasonal water temperature fluctuation and availability of food. Xanthine dehydrogenase does not show significant seasonally related variation, at either location. One cytoplasmic malate dehydrogenase phenotype, that designated 3A, shows significant seasonal variation at Pretty Marsh. The phosphatases and esterases show considerable variation among individuals. Some of this variability can be attributable to nutritional status. As presented in Results, the phosphatases are discussed in two ways: first, in terms of the numbers of individuals displaying visible activity, and second, in terms of the numbers of different phenotypes.

(4) Seasonal Variation In Xanthine Dehydrogenase.

Results show that no additional phenotypes appear over the seasons studied at either Blue Hill Falls or at Pretty Marsh. The relative frequencies of the two XDH phenotypes are consistent with those observed during the study of geographic variation.

At Blue Hill Falls, the frequency of the Rf .36-.40 phenotype remains high during all seasons sampled, and a chi-square test indicates no significant differences in frequencies from season to season. Thus, no seasonal variation is apparent. Moreover, no significant correlation of XDH frequencies exists with seasonal change in seawater temperature. While seasonal water temperature changes do not seem to explain the seasonally uniform, high XDH frequency, neither does nutritional status, as noted earlier in the study of the effects of feeding and fasting on isozyme phenotypes. The same phenotypes are found in both fasted and fed animals, and both treatments show the same proportions of phenotypes. Moreover, these proportions are consistent with those found in the study of geographic variation.

Thus, if temperature and nutritional status effect the frequencies of XDH phenotypes, they probably do so in other ways not examined in this work. Moreover, the frequencies of XDH could be determined by other factors not considered in this study.

At Pretty Marsh, the frequency of the Rf .36-.40 phenotype remains low during March and May, shows an increase in August, but decreases again to former levels in October. A chi-square test indicates no significant differences in frequencies among the seasons sampled. No correlation exists with seasonal change in seawater temperature. As at Blue Hill Falls, it is possible that temperature and XDH frequencies relate in other ways not considered in this study.

(5) Seasonal Variation In Malate Dehydrogenase.

The array of MDH phenotypes detected over the seasons studied was the same as that identified in the study of geographic variation (Figure 9). A consideration of the frequencies of the 2 and 3A phenotypes shows that significant seasonal variation in the frequencies of these phenotypes is evident in animals from both Blue Hill Falls and Pretty Marsh. However, a chi-square comparison of the two populations on a season-to-season basis indicates no significant difference between them on the basis of MDH phenotypic frequencies. Both populations show a progressive increase in the frequency of the 3A phenotype from a low in March to a peak in August. This change in frequency closely parallels seasonal water temperature change at both locations, but is strongly correlated ($r = .98$), only at Pretty Marsh. This confirms the relationship of water temperature to the frequency of the 3A phenotype observed in the study of geographic variation. Nutritional status does not

appear to effect the frequencies of the 2 and 3A MDH phenotypes. In the feeding-fasting experiment, only the 2, 3A, 3B phenotypes were detected (Figure 39). The other phenotypes noted earlier, were not observed. This is attributed to the relative infrequency of these phenotypes, and the small experimental sample size. Despite this, no significant differences in phenotypic frequencies between fed and fasted treatments was observed. It thus seems likely that seawater temperature is a major influence in somehow effecting the frequency of the MDH 3A phenotype.

(6) Seasonal Variation In The Phosphatases: Introductory Statement.

The phosphatases represent a large number of isozymes which catalyze a wide variety of reactions, but all are specific for the phosphomonoester linkage. One group acts at acid pH's, another group functions at alkaline pH's. In rather general ways, the functioning of the phosphatases may reflect broad aspects of metabolism, which in turn may be related to seasonal change and nutritional condition. Ide and Fishman (1969) have demonstrated the association of acid phosphatases with biological membranes, and with lysosomes in particular. Desai (1969) showed in rats starved up to 120 hours, that both free and total acid phosphatase activity increased progressively during the starvation period. During a post starvation re-feeding period enzyme activities returned to normal levels. Thus, acid phosphatase activity seems to be involved in cellular autophagy, possibly as an adaptation to long periods of food deprivation. Sea-stars are known to be able to survive for long periods of time without food, and it may be that such an adaptation to fasting exists in these animals. Alkaline phosphatase, on the other hand, seems to be associated with growing

tissues. For example, high levels of alkaline phosphatase activity are found in the developing human placenta, mammary gland tissue during pregnancy, and the rapidly growing tissues of carcinomas, (Stadtman, 1961). It therefore seems reasonable to suppose that acid and alkaline phosphatase activities in Asterias forbesi in some way relate to seasonal changes in caecal indices which in turn relate to periods of feeding and fasting.

(7) Seasonal Variation In The Presence Of Acid And Alkaline Phosphatase.

The following discussion of the phosphatases will consider the presence of acid and alkaline phosphatase in conjunction with one another, on a season-by-season basis at each location. An analysis of the phosphatases, based on presence or absence of the enzymes in individual specimens of Asterias forbesi, shows three things: first, at a given location, for a particular enzyme system there are differences among seasons (Figures 15 and 16). Second, at any given season there are differences in the frequencies of individuals showing acid and alkaline phosphatase activity at both locations (Figures 15 and 16). Third, at certain seasons differences in the frequencies of individuals showing acid and alkaline phosphatase activity exist between the two populations (Figures 15 and 16).

During March the number of individuals from the salt pond at Blue Hill Falls showing alkaline phosphatase activity is low, but is high at Pretty Marsh. If as natural history studies have suggested, Asterias forbesi is still at a relatively low metabolic point in March, then the numbers of individuals showing alkaline phosphatase activity at any given location is expected to be low. This

is the case at the salt pond, but not so at Pretty Marsh. The differences in alkaline phosphatase activity between the two populations in March may be due to unusual circumstances: the animals collected from Pretty Marsh were obtained from the underside of a floating dock. Despite $0^{o}C$ water temperatures, the animals here were physically (and presumably metabolically) active, and many were observed to be engaged in feeding. This situation is attributed to the fact that the underside of the dock was sheltered and warm due to the absorption of solar energy. Animals collected from the salt pond were extremely inactive and showed no evidence of feeding behavior. The animals appeared to be at a generally low metabolic level. This evidence suggests an association between feeding behavior and the presence of alkaline phosphatase.

During March the number of individuals from Blue Hill Falls showing acid phosphatase activity is relatively high, but is low at Pretty Marsh (Figures 15 and 16). Thus, it seems that where animals were actively feeding as at Pretty Marsh, the number of individuals showing acid phosphatase activity is low, and that where animals were not engaged in feeding as at Blue Hill Falls, the number of individuals displaying acid phosphatase activity is comparatively high.

In May, the number of individuals showing alkaline phosphatase activity is maximum at both Blue Hill Falls and at Pretty Marsh (Figures 15 and 16). This increase in the number of individuals showing alkaline phosphatase activity corresponds to an observable increase in physical activity and feeding behavior in both populations. It corresponds as well to seasonal increase in the

magnitude of hepatic indices of Asterias forbesi from north of Cape Cod, as noted by Sherman (1966).

During May, the number of individuals showing acid phosphatase activity at both Blue Hill Falls and Pretty Marsh is nonexistent. This may reflect a shift in metabolism away from the catabolic activities associated with fasting.

Natural history studies by Galtsoff and Loosanoff (1939), Sherman (1966), and MacKenzie (1969) indicate that during August, Asterias forbesi becomes inactive and ceases to feed. Such activity was noted as well in animals at Blue Hill Falls and at Pretty Marsh. This decrease in activity corresponds with a decline in the number of individuals showing alkaline phosphatase activity and a coincident increase in the number of individuals showing acid phosphatase activity in August. The general decrease in physical activity may be due to the effect of higher water temperature as noted by MacKenzie (1969). While absolute numbers of individuals displaying alkaline phosphatase activity are approximately equal at both locations, the number of individuals showing acid phosphatase activity is higher at Pretty Marsh than at the salt pond. If fasting and the function of acid phosphatase are related, then this difference between the two locations could be attributable to a decrease in feeding behavior and/or to a lack of available food.

In October, as seawater temperatures drop, Asterias forbesi has been shown to be physically active once again and to engage in feeding (Galtsoff and Loosanoff, 1939). If feeding behavior and the number of individuals showing alkaline phosphatase activity are positively correlated, then increased feeding behavior should result

in a corresponding increase in the number of individuals showing activity. Increased feeding behavior in October should result in an increase in the number of individuals showing alkaline phosphatase activity. At Blue Hill Falls, this does not seem to be the case. In fact, a decrease in the number of individuals compared to August was noted. If a particular temperature range is critical to feeding behavior, then it is possible that due to the small size of the salt pond, that the critical temperature is reached earlier than at Pretty Marsh. A coincidental peak in the number of individuals displaying alkaline phosphatase activity could then have been achieved prior to the October sampling date.

At Pretty Marsh, increased feeding behavior corresponds to an increase in the number of individuals displaying alkaline phosphatase activity.

In October, the number of individuals displaying acid phosphatase activity is low at Blue Hill Falls, lower than at Pretty Marsh, and is consistent with expectations as relates feeding behavior. Thus, increased feeding behavior in October should result in a decrease in the number of individuals showing acid phosphatase activity. This seems to be the case. At Pretty Marsh, the number of individuals showing acid phosphatase is less than in August. Such a decrease is again coincidental with an increase in feeding behavior.

(8) Seasonal Variation In Alkaline Phosphatase Phenotypes.

A second approach to the analysis of the phosphatases involves the enumeration and comparison of phenotypes season by season, as was done with XDH and MDH. A consideration of the

alkaline phosphatase phenotypes, insofar as they can be identified, shows two things: First, at both Blue Hill Falls and Pretty Marsh the number of phenotypes is low in March, rises to a maximum in May, then decreases to equivalent levels in August and October (Table 12). Thus, some seasonal variation in phenotypic diversity appears evident at each location. The populations do not differ significantly on the basis of the number of phenotypes observed during each season. The second point is that in May at both Blue Hill Falls and Pretty Marsh, there appear individuals which possess bands of low mobility, in the range of Rf .18 to Rf .33. Individuals possessing these bands of low mobility are extremely infrequent in March, August, and October, in both populations. If, as Powell (1976) has noted, the function of alkaline phosphatase is somehow effected by external substrates, then it is possible that the presence or absence of food might influence the number and variety of alkaline phosphatase phenotypes. Results of the feeding and fasting experiment, shown in Figure 40, indicate two things: First, that feeding and/or fasting does not significantly affect the number of different phenotypes in each treatment group. Second, that one observable effect of fasting seems to be the elimination of slow moving forms of the enzymes, those which show mobilities of Rf .18 and .33. Possibly, the low frequency of these slow moving bands in March, August, and October at Blue Hill Falls and Pretty Marsh is attributable in part to some degree of fasting by the individuals in those locations. Likewise, the presence in May at both locations of bands with Rf values approximating .18 and .33 may be partially attributable to physiological conditions caused by the presence of food materials.

(9) Seasonal Variation In Acid Phosphatase Phenotypes.

A consideration of acid phosphatase phenotypes shows two things: First, at both Blue Hill Falls and Pretty Marsh, the number of phenotypes is low in March, decreases to 0 in May, rises to a maximum in August, and decreases somewhat in October (Table 20). A second point is that there do not seem to be patterns of variation involving specific phenotypes or groups of particular bands, at either location. The populations do not appear to differ significantly on the basis of the number of phenotypes observed during each season. If the function of acid phosphatase is somehow affected by the presence or absence of nutrient material, then possibly some relationship exists between the biochemical diversity of acid phosphatase and seasonal periods of feeding and fasting. No acid phosphatase activity is detectable during May when feeding activity is extremely obvious. On the other hand, acid phosphatase diversity is greatest in August when feeding behavior is reduced or nonexistent. It may be that the increase in phenotypic diversity in August represents an increase in the number of biochemical pathways possibly associated with a metabolic shift toward catabolic processes related to fasting. Unfortunately, no data for acid phosphatase is available from the feeding-fasting experiment.

It is difficult to say exactly what physiological mechanism in these sea-stars is responsible for the differences in the number of phosphatase isozymes observed. Ide and Fishman (1969) have reported that digestive processes themselves are able to change the properties of enzymes. Specifically, the action of other enzymes may transform a given enzyme into another type, having different heat

TABLE 21

Seasonal Variation In The Number Of Acid Phosphatase Phenotypes Observed At Blue Hill Falls And Pretty Marsh

Season	Number of Phenotypes	
	Blue Hill Falls	Pretty Marsh
March	2	1
May	0	0
August	5	5
October	2	4

stability properties, and different pH optima. For example, the action of neuramidase greatly reduces the anodal migration of alkaline phosphatase. The action of hyaluronidase of B glucuronidase changes properties such as those above, and increases anodal migration in starch gels.

(10) Seasonal Variation In The Non-Specific Esterases.

The non-specific esterases represent a large group of variable substrate enzymes which catalyze a variety of reactions, all involving the ester linkage. Results of electrophoretic analysis of esterases at Blue Hill Falls and Pretty Marsh show four things: First, in both populations there exists a baffling array of esterase phenotypes. Second, there exists in both populations a progressive seasonal increase in the number of esterase phenotypes, from a low in March to a maximum in October (Table 13). The reasons for this progressive increase are not known at this time. The third thing is the existence in both populations of two groups of particular esterase bands. The group I isozymes include three bands of Rfs .45, .57, and .64. The group II isozymes include two bands of Rfs .64 and .68. Individual animals can be identified on the basis of the presence or absence of group I and/or group II esterase bands. Fourth, seasonal variation exists in terms of the relative numbers of individuals displaying these particular groups of esterase bands. Moreover, differences exist between Blue Hill Falls and Pretty Marsh at certain seasons, based on the number of individuals displaying these groups of bands.

During March at Blue Hill Falls, both group I and group II isozymes are not detectable by electrophoresis. If, as has been

suggested earlier, animals in March are at a metabolic low point, then this condition could be reflected in the absence of these esterase band groups. In May, the number of individuals showing the group I enzymes is maximal (75%) while the number of individuals showing group II enzymes remains low. In August, no individuals appear to display group I enzymes, while a large number (75%) of individuals show the presence of the group II isozymes. During October the number of individuals showing group I enzymes increases to about 25%, while the number of individuals showing group II enzymes decreases to about 25%. The above results, especially for May and August, suggest a relationship between periods of feeding and fasting, and the relative frequencies of group I and group II esterase isozymes. Specifically during May, a feeding period, there is a high frequency of individuals showing group II enzymes, and a low frequency of individuals having group I enzymes. In August, a fasting period, the frequency of individuals with group I enzymes is high, and the frequency of individuals with group II enzymes is low. Thus, it is possible that the presence of group II enzymes is associated with feeding and the presence of group I enzymes with fasting.

At Pretty Marsh, there exists the same general pattern of seasonal variation in the relative frequencies of the group I and group II enzymes, as has been observed at Blue Hill Falls. However, differences exist between the two populations at certain seasons, notably during May and October. In May, at Blue Hill Falls, the frequency of individuals showing group II enzymes is high (75%) but lower (15%) at Pretty Marsh. No group I enzymes are present at this time in either population. In October, at

Blue Hill Falls, the frequency of individuals showing group II enzymes is about 25% and the frequency of group I enzymes is likewise about 25%. At Pretty Marsh in October, the frequency of individuals showing group II enzymes is lower (8%) than at Blue Hill Falls (25%). The frequency of the group I enzymes is higher (35%) at Pretty Marsh than at Blue Hill Falls (25%). If the frequencies of these two groups of enzymes are related to feeding behavior, as has been suggested earlier, then it is possible that the seasonal differences between populations noted above are somehow related to differences in the availability of food at each location.

If, as Powell (1976) has suggested, the function of the esterases is somehow affected by external substrates, then it is possible that the presence or absence of food could in part determine the frequency and variety of esterase bands. Results of the effects of feeding and fasting on esterase banding patterns, shown in Figures 42 and 43, shows two things: First, only two phenotypes are observed in the fed treatment group, while eight phenotypes are observed in the fasted treatment group. Second, the most common banding pattern in the fed treatment group is that characteristic of the group I esterases noted earlier, i.e., that complex of bands having Rfs of .45, .57, and .64. In the fasted treatment group, all individuals possess the band of Rf .64, but in about 50% of the animals an additional band of Rf .68 appears. This combination of bands of Rf .64 and .68 is equivalent to the group II complex of isozymes noted earlier.

In summary, the association of the presence of group I enzymes with seasonal feeding periods and with the fed treatment

group, and the association of group II enzymes with seasonal fasting periods and with the fasted treatment group, suggest that nutritional status in part may explain some of the seasonal variation observed in the esterases.

As with the phosphatases, it is difficult to say exactly what causes the differences in banding patterns between fed and fasted treatments. Oxford (1975) has studied esterase phenocopies in the snail Cepaea nemoralis. He found that diet could influence the number and mobility of esterase isozymes detectable in hepatic tissues of this snail. In particular, Oxford found that the ingestion of the nettle Urtica dioica L. would induce esterase zones in laboratory reared animals. In Asterias forbesi, one effect of fasting seems to be the resolution of the .64 band into two bands of Rf values .64 and .68. Oxford (1975) has suggested the possibility of post-tranlational modification of primary gene products as a source of additional isozyme forms. Such a phenomenon might well explain the presence of the more anodally moving .68 esterase isozyme. Such a secondary modification might well result in the increase in negative charge on the enzyme molecule by deamidation of a vulnerable glutamine or asparagine residue, or to the blocking of an amine group by formylation or acetylation. That the latter can happen has been demonstrated by Terhorst et al (1973).

Finally, the variety of esterase phenotypes observed in this study has some significance in terms of the biochemical taxonomy of Asterias forbesi. Schopf and Murphy (1973) have characterized Asterias forbesi as possessing a single phenotype of four bands, which distinguishes it from Asterias vulgaris, which has three bands. The large amount of seasonally related individual variation

observed in my study is in disagreement with the findings of Schopf and Murphy (1973).

Leucine aminopeptidase was examined for seasonal variation in both populations. No recognizable pattern of seasonally related phenotypic or genotypic variation was evident. Staining and grouping patterns suggest that the least anodally moving locus is polymorphic and displays two non-linked codominant alleles. Genotypically, heterozygotes are extremely numerous in both populations. Heterosis is suggested as a possible reason for the existence of so many heterozygotes in each population (Wallace, 1968). At Blue Hill Falls, allele frequencies show significant deviations from Hardy-Weinberg expectations during all four sampling seasons. Such deviations could be due to a combination of factors including migration, nutrition, selection, gene flow, and genetic drift. At Pretty Marsh, the population is in Hardy-Weinberg equilibrium during all seasons except August. The deviation noted in August could be caused by any or all of the factors noted above. While the genetic interpretation of LAP presented here is consistent with the findings of Schopf and Murphy (1973), the small sample size and the disproportionately large number of heterozygotes necessitates the re-examination of these two populations for LAP polymorphism.

SUMMARY AND CONCLUSIONS

Geographically separated populations of Asterias forbesi vary on the basis of morphological and protein characters. Morphologically, the massiveness of the skeleton as estimated by carinal ossicle weights varies significantly with geography. Animals with lighter skeletons are found north of Cape Cod, while animals with heavier skeletons are found south of Cape Cod. One explanation suggests that ossicle variation is due to growth as it relates to available foraging time at a critical feeding temperature range. A second explanation assumes a genetic origin for ossicle variation. Based on this assumption, diversifying selection is suggested as a means for accounting for regional differences north and south of Cape Cod.

The populations of animals at Blue Hill Falls and at Merigomish Beach are unique in terms of the weights of carinal ossicles of the animals found in these locations. At Blue Hill Falls, the mean weight of carinal ossicles is extremely low, given the latitude of that population. The range of variation around that low mean weight is the narrowest observed throughout the collection range. A number of factors, including those immediately effecting growth, as well as factors such as founder effect, genetic drift, reduced gene flow selection, and possible hybridization are suggested as reasons for this condition. At Merigomish Beach, the range of variation of ossicle weights is more similar to the range of variation of animals south of Cape

Cod, possibly reflecting certain similarities in the environments of Merigomish Beach and the Cape Cod and Long Island Sound regions.

Biochemically, both xanthine dehydrogenase and malate dehydrogenase show evidence of geographically-related variation. Xanthine dehydrogenase is represented in all populations by two phenotypes. One phenotype, that with bands of Rf .36-.40, display a clinal shift, most evident between Beaufort, North Carolina and Blue Hill Falls. The frequencies of the .36-.40 phenotype show a significant positive correlation with seawater temperature range at each location.

The populations of animals at Blue Hill Falls and Merigomish Beach display unusual features with respect to XDH phenotypic frequencies. At Blue Hill Falls, the frequency of the .36-.40 phenotype is the highest (90%) noted in any population examined. This could be due to such factors as reduced gene flow, genetic drift, selection, and founder effect. At Merigomish, the frequency of the .36-.40 XDH phenotype "fits" the cline best at a point south of Cape Cod. As with ossicle weights, this may reflect similarities of environments at Merigomish and the Long Island Sound region.

Interpretation of geographic variation in malate dehydrogenase is difficult. This enzyme system appears to be represented by six bands of differing mobilities. These six bands are combined to give seven phenotypic classes, which show broad regional patterns of distribution. The two MDH phenotypes designated as 2 and 3A are found in varying frequencies in all populations. They are found exclusively in those populations at the extremes of the collection transect, that is, at Beaufort, North Carolina and at St. Ann's Bay, Nova Scotia. The remaining MDH phenotypes are re-

stricted to more centrally located populations. Some clinal variation in the frequency of the 3A MDH phenotype is evident and correlates positively with mean annual seawater temperature at each location.

A study of seasonal variation in isozyme banding patterns at Blue Hill Falls and at Pretty Marsh shows no significant variation in the frequencies of XDH phenotypes, at either location. The frequency of the 3A MDH phenotype shows significant seasonal variation at both Blue Hill Falls and Pretty Marsh. The 3A frequency correlates positively with seasonal change in seawater temperature at Pretty Marsh, but not at Blue Hill Falls. Banding patterns of the phosphatases and esterases show significant seasonal variation at each location. The frequency of certain banding patterns, particularly in the esterases, seems to be associated with seasonal periods when Asterias forbesi actively feeds, and ceases to feed. A laboratory experiment, testing the effects of feeding and fasting on isozyme banding patterns, shows the existence of certain groups of bands characteristic of each treatment. These groups of bands respectively coincide with those bands identified during periods of seasonal feeding and fasting. Thus, it appears that some seasonal variation in esterase banding patterns is attributable to nutritional status.

No evidence of seasonal variation in the frequencies of leucine aminopeptidase phenotypes was observed either at Blue Hill Falls or at Pretty Marsh. However, LAP shows evidence of polymorphism at one locus. The Blue Hill Falls population shows significant deviation from Hardy-Weinberg expectations during all four sampling seasons, while the Pretty Marsh population is

in Hardy-Weinberg equilibrium three out of the four seasons examined. An excess of heterozygotes is observed at each location. Technical reasons deem it necessary that this enzyme system be re-examined.

APPENDIX

APPENDIX

A. Formulas For The Preparation Of Electrophoresis Buffers

(1) Tris-borate EDTA, .1M, pH 8.9 - Buffer System I.

(a) Tris	122.4	g
(b) Boric acid	7.7	g
(c) EDTA	5.6	g
(d) H_2O	1	liter

(e) Adjust pH to 8.9

(f) Dilute 9:1 with distilled water

(2) Tris-borate, .1M, pH 8.9 - Buffer System II.

(a) Tris	122.4	g
(b) Boric acid	7.7	g
(c) H_2O	1	liter

(d) Adjust pH to 8.9

(e) Dilute 9:1 with distilled water

B. A Formula For The Preparation Of Polyacrylamide Gels

(1)	30% Cyanogum 41 (Sigma) stock solution in .1M Tris-borate EDTA pH 8.9 or .1M Tris-borate pH 8.9	40 ml
(2)	.1M Tris-borate EDTA or .1M Tris-borate pH 8.9	160 ml
(3)	Tetramethylethylenediamine (TMED) (Sigma)	1.8 ml
(4)	Ammonium persulfate 10%	.43 ml

Gels prepared for LAP and AlkP required the addition of 10.7 ml of .1M $MgCl_2$ to the gel mixture. Three millimeter thick gels required about 145 ml of gel solution. Six millimeter gels required about 290 ml of gel solution.

C. Techniques For The Detection Of Enzymes

(1) Xanthine dehydrogenase XDH.

Following electrophoresis, gels were placed in 10 x 6 x 1 3/4" Pyrex baking dishes, and incubated in the dark with mild agitation for one hour at room temperature in the following solution:

.01 M Tris-HCl buffer pH 7.5	100 ml
.05 M Hypoxanthine in .1 N NCl	12 ml
NAD	30 mg
Nitro blue tetrazolium	80 mg
1 M KCN	0.2 ml
Phenazine methosulfate (PMS) 2 mg/ml	1 ml

(2) Malate dehydrogenase MDH.

After electrophoresis, gels were placed in Pyrex containers and incubated in the dark with mild agitation for one hour at room temperature in the following solution:

Distilled water	80 ml
.1 M DL Malic acid	10 ml
1 M Tris-HCl buffer pH 8.5	10 ml
1 M KCN	1 ml
NAD	15 mg
Nitro blue tetrazolium	50 mg

After one hour, 1 ml of phenozine methosulfate (PMS), 2 mg/ml was added to the staining mixture. Since MDH staining is rather heavy, MDH gels were scored as band patterns progressively developed.

(3) Alkaline phosphatase (AlkP).

Gels were incubated for 20 minutes in 50 ml of cold .01 M Tris-HCl pH 9.2 at 4^{o}C, then stained with the following mixture at room temperature until bands appeared:

.01 M Tris-Hcl pH 9.2	50 ml
Na α Napthyl phosphate	100 mg
Fast Blue BB	100 mg
.1 M $MgCl_2$	0.25 mg
10% $MnCl_2$	0.25 ml
20% NaCl	5 ml
Polyvinyl pyrollidone	0.25 g

(4) Acid phosphatase (AcP).

Procedures for acid phosphatase were the same as for alkaline phosphatase except that 0.1 M acetate buffer pH 5.0 was used instead of .01 M Tris-Hcl pH 9.2.

(5) Non-specific esterases (EST).

Following electrophoresis, gels were incubated for ½ hour in cold .5 M boric acid at 4°C. The gels were then washed in distilled water and stained in a mixture of the following solutions:

Solution A

50% acetone	1 ml
α naphthyl acetate	40 mg

Solution B

.1 M phosphate buffer pH 6.5	100 ml
Fast Red TRN	100 mg

The gels were incubated at room temperature until bands appeared, usually within one hour.

(6) Glutamate oxaloacetate transaminase (GOT).

Modified from Smith. The gels were incubated for 20 minutes at room temperature in the following mixture:

.2 M Tris-HCl pH 8.0	80 ml
Pyridoxal-S-phosphate 1 mg/ml	1 ml
L Aspartic acid	400 mg
α Ketoglutaric acid	200 mg

After twenty minutes, 200 mg/ml of Fast Blue BB, dissolved 20 ml of .2M Tris-HCl pH 8.0 was added to each gel. The gels were allowed to incubate until bands appeared, usually within one hour.

(7) Leucine aminopeptidase (LAP).

Upon completion of electrophoresis, gels were incubated at $4^{o}C$ for ½ hour in cold .5M boric acid, then rinsed with distilled water and stained with a mixture of the following solutions:

Solution A

50% Acetone	1 ml
L-leucyl-B-naphthylamide HCl	40 mg

Solution B

.2 M Tris maleate buffer pH 5.2	100 ml
Fast Black K	100 mg

Gels were incubated in a mixture of these two solutions at room temperature until bands appeared.

REFERENCES

REFERENCES

Agassiz, A., 1877. North American Star Fishes. Mem. Mus. Comp. Zool. 5(1):1-136.

Aldrich, F.A., 1956. A comparative study of the identification characters of Asterias forbesi and Asterias vulgaris. Notul. Nat. 285:1-3.

Ayala, F.J. and J.W. Valentine. 1974. Genetic variability in the cosmopolitan deep water ophiuran Ophiomusium lymani. Mar. Biol. 27:51-57.

Bousfield, E.L. and M.L.H. Thomas. 1975. Postglacial changes in the distribution of littoral marine invertebrates in the Canadian Atlantic region. Proc. N.S. Inst. Sci. 27(3): 47-60.

Bradshaw, A.D., 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genetics 13.

Carr, A. 1981. Personal communication.

Cerame-Vivas, M.J. and I.E. Gray. 1966. The distributional pattern of benthic invertebrates off the continental shelf of North Carolina. Ecology 47:260-270.

Chaet, A.B. and R.A. McConnaughy. 1959. Physiologic activity of nerve extracts. Biol. Bull. 117:407.

Clark, H.L., 1904. The echinoderms of the Woods Hole Region. Bull. U.S. Fish. Comm. for 1902, 22:545-576.

Coe, W.R., 1912. Echinoderms of Connecticut. Bull. Conn. St. Geol. Nat. Hist. Surv. 19.

Desai, I.D., 1969. Regulation of lysosomal enzymes. I. Adaptive changes in enzyme activities during starvation and refeeding. Can. J. Biochem. 47:785-790.

Dobzhansky, Th., A.S. Hunter, O. Pavlovsky, B. Spassky, and B. Wallace. 1963. Genetics of natural populations XXXI. Genetics of an isolated marginal population of Drosophila pseudoobscura. Genetics 48:91-103.

Drinkwater, K., 1981. Personal communication.

Ebert, T., 1980. Relative growth of sea-urchin jaws: an example of plastic resource allocation. Bull. Mar. Sci. 30(2): 467-474.

Endler, J. 1977. Geographic Variation, Speciation and Clines. Princeton Univ. Press, Princeton, N.J., 246 pp.

Ernst, E.J. 1967. The distribution, ecology, environmental behavior and possible hybridization of the sea stars Asterias forbesi(Desor) and Asterias vulgaris Verrill in the subtidal zone of Long Island. Ph.D. Thesis. New York Univ. 1967. Univ. Microfilms, Inc., Ann Arbor, Michigan.

Franz, D.R., E.K. Worley, and A.S. Merrill. 1981. Distribution patterns of common sea-stars of the middle Atlantic continental shelf of the northwest Atlantic (Gulf of Maine to Cape Hatteras). Biol. Bull. 160:395-418.

Galtsoff, P. and V. Loosanoff. 1939. Natural history of Asterias forbesi. Bull. U.S. Bur. Fish. 49(31): 75-132.

Gemmill, J. 1914. The development and certain points in the adult structure of Asterias rubens. Philos. Trans. Ray. Soc. London 205B, p 213.

Gosner, K.L. 1971. Guide to Identification of Marine and Estuarine Invertebrates. Wiley-Interscience. New York. 693pp.

Hettler, W. 1981. Personal communication.

Hochachka, P.W. and G.N. Somero. 1973. Strategies of Biochemical Adaptation. W.B. Saunders Co., Phil. 368 pp.

Hoffman, R.J. 1981. Evolutionary genetics of Metridium senile. II. Geographic patterns of allozyme variation. Biochemical Genetics 19(1 & 2).

Hubby, J.L. and R.C. Lewontin. 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in Drosophila pseudoobscura. Genetics 54:577-594.

Huntsman, A. and M. Sparks. 1924. Limiting factors for marine animals. III. Contrib. Canad. Biol. Ser. 2.

Hyman, L.H. 1955. The Invertebrates: Echinodermata The Coelamate Bilateria. Vol. IV. New York. McGraw-Hill Book Co., 763 pp.

Ide, H. and W.H. Fishman. 1969. Dual localization of β glucuronidase and acid phosphatase in lysosomes and in microsomes. II. Membrane associated enzymes. Histochemie 20:300-321.

Johnson, C.W. 1934. List of marine mollusca of the Atlantic coast from Labrador to Texas. Proc. Bostn. Soc. Nat. Hist. 40: 1-204.

Johnson, G.B. 1974. Enzyme polymorphism and metabolism. Science 184:28-37.

Koehn, R. 1969. Esterase heterogeneity: dynamics of a polymorphism. Science 163:943-944.

Lewontin, R. 1975. Personal communication.

Lewontin, R. and J.L. Hubby. 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudoobscura. Genetics 54: 595-609.

MacKenzie, C.L. 1969. Feeding rate of starfish, Asterias forbesi (Desor) at controlled water temperatures and during different seasons of the year. Fish. Bull. 68(1):67-72.

Marcus, N. 1977. Genetic variation within and between geographically separated populations of the sea-urchin Arbacia punctulata. Biol. Bull. 153:560-576.

Matsuoka, N. 1981. Phylogenetic relationships among five species of starfish of the genus Asterina: an electrophoretic study. Comp. Biochem. Physiol. 703:739-743.

Mayr, E. 1975. Population, Species, and Evolution. The Belknap Press of Harvard Univ. Press, Cambridge, Ma. 453 pp.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press of Harvard Univ. Press, Cambridge, Ma.

Mead, A.D. 1900. On the correlation between growth and food supply in the star-fish. An. Nat. 34:17-23.

Menge, B. 1979. Coexistence between the sea-stars Asterias vulgaris and Asterias forbesi in a heterogeneous environment: a non-equilibrium explanation. Oecol. 41:245-272.

Mileikovsky, S.A. 1971. Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Mar. Biol. 10:193-213.

Oxford, G.S. 1975. Food induced esterase phenocopies in the snail Cepaea nemoralis. Heredity 35:361-370.

Perlmutter, A. and R.F. Nigrelli. 1960. A possible hybrid population of the starfish Asterias forbesi (Desor) in western Long Island Sound. Proc. Nat. Shellfish Assoc. 51:93-96.

Pesch, G. 1974. Protein polymorphism in the hard clam Mercenaria mercenaria and Mercenaria campechiensis. Biol. Bull. 146: 393-403.

Powell, J.R. 1976. Protein variation in natural populations of animals. Chap. 2. In: T. Dobzhansky, M. Hecht, and W. Steere, Eds., Evolutionary Biology 8. Plenum Press, New York.

Prakash, S., R.C. Lewontin, and J.L. Hubby. 1969. A molecular approach to genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal, and isolated populations of Drosophila pseudoobscura. Genetics 61: 841-858.

Pyle, R.L. 1962. Serial Atlas of the Marine Environment: Folio #1. Sea Surface Temperature Regime in the Western North Atlantic. 1953-1954. American Geographical Society.

Roberts, R.M. and J.S. Jones. 1972. Improved apparatus for vertical gel electrophoresis. Anal. Biochem. 49:592-597.

Rutherford, J.C. 1977. Geological variation in morphological and electrophoretic characters in the holothurian Cucumaria curata. Mar. Biol. 43:165-174.

Schick, J.M. W.F. Taylor, and A.N. Lamb. 1981. Reproduction and genetic variation in the deposit feeding sea-star Ctenodiscus crispatus. Mar. Biol. 63:51-66.

Schopf, T.J.M. and J.L. Gooch. 1971. Gene frequencies in a marine ectoproct: a cline in natural populations related to sea temperature. Evolution 25:286-289.

Schopf, T.J.M and L.S. Murphy. 1973. Protein polymorphism of the hybridizing sea-stars Asterias forbesi and Asterias vulgaris and implications for their evolution. Biol. Bull. 145:587-597.

Shaw, C.R. and Rupi Prasad. 1969. Starch gel electrophoresis of enzymes. A compilation of recipes. Biochem. Genet. 4:297-320.

Sherman, V. 1966. The reproductive biology of selected echinoderms from Cape Cod, Ma. M.S. Thesis, Univ. of California, Los Angeles.

Sherman, V. 1971. Personal communication with Prof. J.M. Anderson.

Smith, G. 1940. Factors limiting distribution and size in the starfish. Jour. Fish. Research Bd. Canada 5.

Stadtman, T. 1961. Alkaline phosphatases. Chap. 4. In: Boyer, P., H. Lardy, and K. Myrback, Eds., The Enzymes 5, 645 pp.

Swan, E. 1975. Personal communication.

Terhorst, C.P., Moller, W., Laursen, R. and Wittman-Liebold, B. 1973. The primary structure of an acidic protein from 50-S ribosomes of Escherichia coli which is involved in GTP hydrolysis dependent on elongation factors G and T. Eur. J. Biochem. 34:138-152.

Thurburg, F. 1981. Personal communication.

U.S. Department of Commerce. 1951. Coast and Geodetic Survey Special Publication No. 278.

Vadas, R. 1981. Personal communication.

Volk, J. 1979. Personal communication.

Walford, L. and R.I. Wickland. 1968. Monthly Sea Temperature Structure from the Florida Keys to Cape Cod. Serial Atlas of the Marine Environment No. 15, Folio 1-2. American Geographical Society, New York.

Walker, C.W. 1980. Spermatogenic columns, somatic cells and the microenvironment of germinal cells in the testes of asteroids. Jour. Morph. 166:81.

Walker, C.W. 1973. Morphology and histology of the reproductive systems of Asterias vulgaris. Appendix I. Master's Thesis. Cornell University, 135 pp.

Wallace, B. 1968. Topics in Population Genetics. W.W. Norton and Company Inc. New York. 481pp.

Weiler, J.D.M. and J.R. Keeley. 1980. Monthly sea surface temperature from the Gulf of St. Lawrence. Gov. of Canada Marine Environmental Data Service Technical Report No. 7.

Zar, J. 1974. Biostatistical Analysis. Prentice-Hall Inc., Englewood Cliffs, N.J. 620pp.